



**Memorandum**

**DEC 22 2003**

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Date: **DEC 22 2003**  
From: Division of Dietary Supplement Products, Office of Nutritional Products,  
Labeling and Dietary Supplements, HFS-810  
Subject: 75-Day Pre-market Notification of New Dietary Ingredients  
To: Dockets Management Branch, HFA-305

Subject of the Notification: *Hoodia gordonii*  
Firm: Goen Group  
Date Received by FDA: April 4, 2003  
90-Day Date: July 4, 2003

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day pre-market notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Attachments

95S-0316

RPT186



JUN 19 2003

Ralph Fucetola, JD  
Goen Technologies Corporation  
Legal Department  
8 Ridgedale Avenue  
Cedar Knolls, New Jersey 07927

Dear Mr. Fucetola:

This is to inform you that the notification, dated March 27, 2003, that you submitted pursuant to 21 U.S.C. 350b(a)(2)(section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)) was filed by the Food and Drug Administration (FDA) on April 4, 2003. Your notification identified Hoodia gordonii extract as a substance that you assert is a new dietary ingredient in the TrimSpa® product.

Your notification states that, under the recommended serving and conditions of use, the supplement will be in capsule form. Suggested servings will likely be one to two capsules twenty minutes before each meal. The suggested purpose for use is for appetite suppression.

Under 21 U.S.C. 350b(a)(2), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

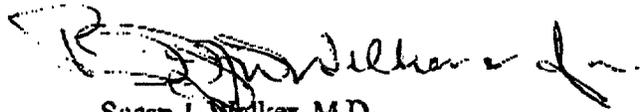
FDA has carefully considered the information in your submission, and the agency has significant concerns about the evidence on which you rely to support your conclusion that a dietary supplement containing Hoodia gordonii extract will reasonably be expected to be safe. In the human study and the other information submitted, it was unclear as to whether the test substances used in the studies are the same as that of the ingredient in your notification. Moreover, your submission provides no information that the test substances used in the referenced studies are qualitatively or quantitatively similar to your ingredient or how these studies are relevant to evaluating the safe use of your ingredient under the recommended conditions of use.

For the reasons discussed above, the information in your submission does not provide an adequate basis to conclude that Hoodia gordonii in the Trimspa® product, when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Your notification will be kept confidential for 90 days after the filing date of April 4, 2003. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number 958-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

If you have any questions concerning this matter, please contact Victoria Lutwak at (301) 436-2375.

Sincerely yours,



Susan J. Walker, M.D.  
Acting Division Director  
Division of Dietary Supplement Programs  
Office of Nutritional Products, Labeling  
and Dietary Supplements  
Center for Food Safety  
and Applied Nutrition

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# GOEN GROUP

LEGAL DEPARTMENT  
8 Ridgedale Avenue  
Cedar Knolls, NJ 07927

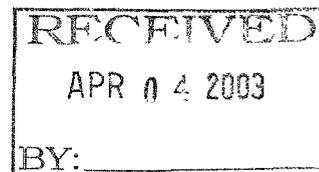
973-267-4400 x 4020

Fax 973-267-6951

LEGAL DEPARTMENT  
Ralph Fucetola, JD  
ralph.fucetola@goengroup.com

March 27, 2003

**Division of Standards and Labeling Regulations**  
**Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)**  
**Center for Food Safety and Applied Nutrition**  
**Food and Drug Administration**  
5100 Paint Branch Parkway  
College Park, MD, 20740-3835



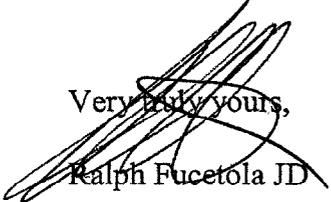
Re: *Hoodia gordonii*

Gentlepeople,

Kindly accept the within Notice under 21 CFR 190.6, with attachments, and acknowledge the receipt thereof. The enclosed includes:

- A. Notice
- B. Ingredient List
- C. Attachments:
  1. Article from South Africa, Dispatch Online, of May 3, 2002
  2. Phytopharm December 5, 2001 Press Release
  3. The FASEB Journal, Abstracts 2.1-537.42(338.5) – March 7, 2001
  4. The FASEB Journal, Abstracts 2.1-537.42(747.3) – March 7, 2001
  5. Patent No. 6,376,657.

Very truly yours,

  
Ralph Fucetola JD

**SECRETARY OF HEALTH AND HUMAN SERVICES  
UNITED STATES OF AMERICA**

**NOTICE OF NEW DIETARY INGREDIENT – 21 CFR 190.6**

Division of Standards and Labeling Regulations  
Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD, 20740-3835

Date: March 27, 2003.

Re: This Notice covers the following Dietary Ingredient: *Hoodia gordonii* Extract.

**NOTICE**

**PLEASE TAKE NOTICE under 21 U.S.C. 350b and 21 CFR 190.6:**

(1) Company name and complete address: **Goen Technologies Corporation**, a New Jersey corporation with offices at 8 Ridgedale Avenue, Cedar Knolls, NJ 07927.

(2) The name of the dietary ingredient is *Hoodia gordonii*.

(3) Attached hereto is a description of the dietary supplement that will contain the dietary ingredient including, (i) the level of the ingredient and (ii) the conditions of use recommended in the labeling

(4) The history of use or other evidence of safety establishing that the dietary ingredient

Attached hereto are several items that substantiate the history of use and safety of the Dietary Ingredient. None of the attachments are proprietary. These are:

1. Article from South Africa, Dispatch Online, of May 3, 2002
2. Phytopharm December 5, 2001 Press Release
3. The FASEB Journal, Abstracts 2.1-537.42(338.5) – March 7, 2001
4. The FASEB Journal, Abstracts 2.1-537.42(747.3) – March 7, 2001
5. Patent No. **6,376,657**.

The South African article sets forth the historical use of the *Hoodia* cactus as a food, by the native San people of the desert regions of South Africa, that suppresses appetite and the work done through the Council Scientific and Industrial Research (CSIR) with Phytopharm and Pfizer to isolate the active ingredient, P57. The Phytopharm press release announces the positive safety evaluation of *Hoodia* extract. The two Abstracts relate to *Effects of Hoodia Plant on Food Intake and Body Weight in Lean and Obese... Rats.* (338.5) and *Mineral Content of Edible Hoodia Plant Species.* They confirm the nutrient value and safety of *Hoodia*. The Patent relates to the extraction of the active ingredient and the use thereof for the support of normal structure and function in appetite suppression and states in its Abstract,

“...an extract obtainable from a plant of the genus *Trichocaulon* or *Hoodia* containing an appetite suppressant agent... A process for obtaining the extract... The invention also extends to the use of such extracts and compound... and its analogues for the manufacture of medicaments having appetite suppressant activity. The invention further provides novel intermediates for the synthesis...”

## CERTIFICATION

The undersigned, being duly authorized by the firm submitting the above Notice, certifies, as of the date first written above: (a) that the information contained in the Notice is complete and accurate, and (b) that the firm has concluded that a dietary supplement containing such Dietary Ingredient will reasonably be expected to be safe.

The undersigned certifies that the above Certification is true and is aware that the undersigned is subject to punishment as for perjury if the Certification is willfully false.

Dated: March 27, 2003

  
Albert M. Fleischner, Ph.D.

Prepared by:  
Ralph Fucetola, JD  
All rights reserved -- UCC 1-207

## TrimSpa® Hoodia gordonii Product

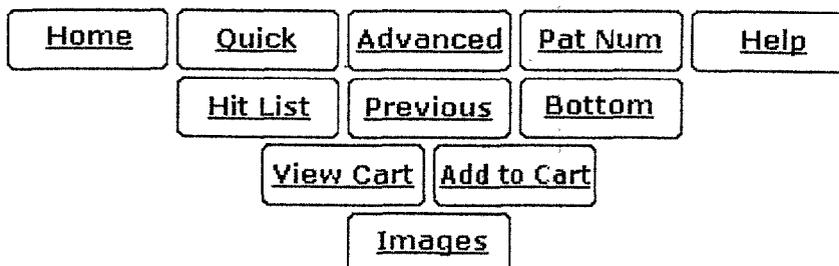
|   |           |      |
|---|-----------|------|
| Hoodia Gordonii   | 75.0 mg   |      |
| Chromium (as chromium Chelavit™ dinicotinate glycinate) | 75.0 mcg  |      |
| Vanadium (as vanadium amino acid chelate)               | 15.0 mcg  |      |
| Glucomannan   | 200.0 mg  |      |
| Sodium Carboxymethyl Cellulose                          | 50.0 mg   |      |
| Citrus Naringinine (46% Naringinine)                    | 5.00 mg   |      |
| Green Tea Extract (40%)                                 | 125.0 mg  |      |
| Coclean™ (10% Theobromine )                             | 162.5 mg  |      |
| Glucosamine   | 50.0 mg   | 1190 |
|   | <hr/>     |      |
|   | 667.59 mg |      |

Other ingredients: dicalcium phosphate, microcrystalline cellulose, croscarmellose sodium, stearic acid, magnesium stearate

$\frac{10}{100}$

Recommended Use: As a dietary supplement, one to two capsules twenty minutes before each meal.

All rights reserved  
Draft 03/27/03

USPTO PATENT FULL-TEXT AND IMAGE DATABASE

( 2 of 2 )

United States Patent  
Van Heerden , et al.

6,376,657  
April 23, 2002

Pharmaceutical compositions having appetite suppressant activity

**Abstract**

##STR1## A pharmaceutical composition which contains an extract obtainable from a plant of the genus *Trichocaulon* or *Hoodia* containing an appetite suppressant agent having the formula (1). A process for obtaining the extract and a process for synthesizing compound (1) and its analogues and derivatives is also provided. The invention also extends to the use of such extracts and compound (1) and its analogues for the manufacture of medicaments having appetite suppressant activity. The invention further provides novel intermediates for the synthesis of compound (1).

Inventors: **Van Heerden; Fanie Retief (Fairland, ZA); Vleggaar; Robert (Pretoria, ZA); Horak; Roelof Marthinus (Pretoria, ZA); Learmonth; Robin Alec (Pretoria, ZA); Maharaj; Vinesh (Pretoria, ZA); Whittal; Rory Desmond (Pretoria, ZA)**

Assignee: **CSIR (Pretoria, ZA)**

Appl. No.: **402962**

Filed: **October 13, 1999**

PCT Filed: **April 15, 1998**

PCT NO: **PCT/GB98/01100**

371 Date: **October 13, 1999**

102(e) Date: **October 13, 1999**

PCT PUB.NO.: **WO98/34624**

PCT PUB. Date: **October 22, 1998**

**Foreign Application Priority Data**

Apr 15, 1997[ZA]

97/3201

**Current U.S. Class:****536/5; 424/725****Intern'l Class:****A61K 039/385; A61K 031/44; C07J 005/00****Field of Search:****536/5 424/195.1 514/278,221,90,303,326**

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| <u>4302477</u> | Nov., 1981 | Mendy et al.     |          |
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Primary Examiner: Gitomer; Ralph

Assistant Examiner: Khare; Devesh

Attorney, Agent or Firm: Morgan, Lewis & Bockius LLP

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### Claims

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What is claimed is:

1. An extract obtainable from a plant of the genus *Trichocaulon* or of the genus *Hoodia* which comprises

an appetite suppressant agent having the formula ##STR72##

2. An extract as claimed in claim 2 wherein the plant of the genus *Trichocaulon* is selected from the species *Trichocaulon piliferum* and *Trichocaulon officinale* and the plant of the genus *Hoodia* is selected from the species *Hoodia curroii*, *Hoodia gordonii* and *Hoodia lugardii*.
3. An extract as claimed in claim 2 wherein substantially all the non-active impurities have been removed.
4. An extract as claimed in claim 1 which has been processed to a free-flowing powder.
5. A composition having appetite suppressant activity comprising the extract as claimed in claim 1.
6. A composition as claimed in claim 5 when admixed with a pharmaceutical excipient, diluent or carrier.
7. A composition as claimed in claim 5, which is prepared in unit dosage form.
8. The use of an extract as claimed in claim 1 in the manufacture of a medicament having appetite suppressant activity.
9. An extract as claimed in claim 1 for use as a medicament having appetite suppressant activity.
10. A method of combating obesity in a human or animal comprising administering to said human or animal an obesity combating amount of an extract as claimed in claim 1.
11. A compound having the structural formula: ##STR73##

in which R=alkyl;

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5 or C5-C6.

12. A compound as claimed in claim 11 wherein there is a bond between C5-C6, R=methyl, R.sub.1 =tigloyl, R.sub.2 =3-0-[-.beta.-D-thevetopyranosyl-(1.fwdarw.4)-.beta.-D-cymaropyranosyl-(1.fwdarw.4)-.beta.-D-cymaropyranosyl], the compound having the structural formula ##STR74##
13. A process of forming a trisaccharide and coupling the resultant trisaccharide to a steroid intermediate, which includes the steps of
  - i) coupling a selectively protected cymarose moiety of formula (40) and compound (45) using tin (II) chloride, AgOTf, Cp.sub.2 ZrCl.sub.2 to produce a compound of the formula ##STR75##

in which Z=TBDMS=t-butyl dimethylsilyl;

  - ii) treating compound (57) with tetrabutylammonium fluoride and diethylaminosulphur trifluoride to produce a trisaccharide compound having the formula ##STR76##

and iii) coupling the trisaccharide of formula (58) with a steroid intermediate of the formula ##STR77##

using tin (II) chloride, AgOTf, Cp.sub.2 ZrCl.sub.2 to produce compound (1) as claimed in claim 12.

14. A composition having appetite suppressant activity comprising a compound as claimed in claim 11.

15. A composition as claimed in claim 14 wherein the compound is the compound of formula (1).

16. A composition as claimed in claim 14 when admixed with a pharmaceutical excipient, diluent or carrier.

17. A composition as claimed in claim 14, which is prepared in unit dosage form.

18. The use of a compound as claimed in claim 11 in the manufacture of a medicament having appetite suppressant activity.

19. The use as claimed in claim 18 of a compound of formula (1).

20. A compound as claimed in claim 11 for use as a medicament having appetite suppressant activity.

21. A compound claim 20 which is the compound of formula (1).

22. A foodstuff or beverage comprising an effective quantity of a compound as claimed in claim 11 to have an appetite suppressant effect when ingested.

23. A foodstuff or beverage as claimed in claim 22 wherein the compound is the compound of formula (1).

24. A compound of formula (1) as claimed in claim 11 isolated from a plant of the genus *Trichocaulon* or from the genus *Hoodia* for use as a medicament having appetite suppressant activity.

25. A compound as claimed in claim 24 wherein the compound is isolated from a plant of the species *Trichocaulon piliferum* or *Trichocaulon officinale* or from *Hoodia currorii*, *Hoodia gordonii* or *Hoodia lugardii*.

26. A compound of claim 11, wherein R.sub.1 is tigloyl.

27. A compound of claim 11 having the following stereochemical structure: ##STR78##

28. A compound having the structural formula ##STR79##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group.

29. A compound of claim 28, wherein R.sub.1 is tigloyl.

30. A compound of claim 28 having the following stereochemical structure: ##STR80##

31. A compound having the structural formula: ##STR81##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group.

32. A compound of claim 31, wherein R.sub.1 is tigloyl.

33. A compound of claim 31, having the following stereochemical structure: ##STR82##

34. A compound having the structural formula: ##STR83##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group.

35. A compound of claim 34, wherein R.sub.1 is tigloyl.

36. A compound of claim 34 having the following stereochemical structure: ##STR84##

37. A compound having the structural formula: ##STR85##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group.

38. A compound of claim 37, wherein R.sub.1 is tigloyl.

39. A compound of claim 37 having the following stereochemical structure: ##STR86##

40. A compound having the structural formula: ##STR87##

in which R=alkyl;

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5 or C5-C6.

41. A compound having the structural formula: ##STR88##

in which R=alkyl;

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5 or C5-C6.

42. A compound having the structural formula: ##STR89##

in which R=alkyl;

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group; and

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5 or C5-C6.

43. A compound having the structural formula: ##STR90##

in which R=alkyl;

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group; and

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5, C5-C6 or C14-C15.

44. A compound having the structural formula: ##STR91##

in which R=alkyl;

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group; and

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5, C5-C6 or C14-C15.

45. A compound having the structural formula: ##STR92##

in which R=alkyl;

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5, C5-C6 or C14-C15; and

R.sub.3.dbd.H, alkyl, aryl, acyl, or glucoxy.

46. A compound having the structural formula: ##STR93##

in which R.dbd.H, alkyl, aryl, or any steroid possessing a C14 beta hydroxy group, a C12 beta hydroxy functionality, a C17 acyl group, a C5-C6 olefin, or combinations thereof.

47. A process of coupling a monosaccharide cymarose to a steroid intermediate, which includes the steps of

i) reacting a cymarose moiety of formula (38) with a steroid intermediate of formula (15) in the presence of tin chloride in a solvent to produce a compound 3-O-[4-O-benzoyl-2-phenylthio-.beta.-D-cymaropyranosyl]-12,14-.beta.-dihydroxy-pregnan-5-ene-20-one of the formula ##STR94##

and (ii) treating the compound (51) with tiglic acid chloride in pyridine and thereafter with a base to produce a compound 3-O-[4-O-benzoyl-2-phenylthio-.beta.-D-cymaropyranosyl]-12.beta.-tigloyl-14.beta.-hydroxy-pregnan-5-ene-20-one of the formula ##STR95##

48. A compound of formula (52) when produced by a process as claimed in claim 47.

49. A process of coupling a monosaccharide cymarose moiety to a monosaccharide thevetose moiety and coupling the resultant disaccharide to the compound of formula (52) as claimed in claim 48 which includes the steps of

i) coupling a selectively protected cymarose moiety of formula (40) and a monosaccharide thevetose moiety of formula (50 A) using tin chloride (SnCl<sub>2</sub>) and silver trifluoromethanesulphonate to produce a compound of the formula ##STR96##

in which Z=TBDMS=t-butyl dimethylsilyl

ii) treating compound (53) with tetrabutylammonium fluoride to produce a compound of the formula ##STR97##

iii) treating compound (54) with diethylaminosulphur trifluoride to produce a compound of the formula ##STR98##

iv) reacting compound (55) with compound (52) as claimed in claim 48 to produce a compound of the formula ##STR99##

and (v) treating compound (56) in a Raney-Nickel reaction and thereafter with a base to produce compound (1) as claimed in claim 12.

50. A composition having appetite suppressant activity comprising a compound of formula (1) isolated from a plant of the genus *Trichocaulon* or of the genus *Hoodia*:

51. A composition as claimed in claim 50 wherein the compound is isolated and/or purified from a plant of the species *Trichocaulon piliferum* or *Trichocaulon officinale* or from of the species *Hoodia curronii*, *Hoodia gordonii* or *Hoodia lugardii*.

52. A composition as claimed in claim 50 wherein the compound is isolated and/or purified from an extract derived from a plant of the species *Trichocaulon piliferum*, *Trichocaulon officinale* or from a plant of the species *Hoodia curronii*, *Hoodia gordonii* or *Hoodia lugardii*.

53. A composition as claimed in claim 50, when admixed with a pharmaceutical excipient, diluent or

carrier.

54. A composition as claimed in claim 53 which is prepared in unit dosage form.

55. A compound having the structural formula ##STR100##

56. A compound having the structural formula ##STR101##

57. A compound having the structural formula ##STR102##

58. A structure of the formula 3-O-.beta.-D-theverosyl-(1.fwdarw.4)-p-D-cymaropyranosyl-(1.fwdarw.4)-.bet a. -D-cymaropyranoside-12.beta.-O-tigloyl-14.beta.-hydroxy-pregnane-5-ene-20- one.

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*Description*

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This application is the U.S. national phase of PCT International Application No. PCT/GB98/01100, filed Apr. 15, 1998, which claims priority to South African application No. 97/3201, filed Apr. 15, 1997.

THIS INVENTION relates to steroidal glycosides, to compositions containing such steroidal glycosides and to a new use for these steroidal glycosides and the compositions containing them. The invention further relates to a method of extracting and isolating these steroidal glycosides from plant material, to a method of synthetically producing these steroidal glycosides, and to the products of such an extraction and such a synthesis process.

In a particular application, the invention relates to an appetite suppressant agent, to a process for synthetically producing the appetite suppressant agent, to a process for extracting the appetite suppressant agent from plant material, to an appetite suppressant composition containing the appetite suppressant agent, and to a method of suppressing an appetite.

According to the invention, there is provided a process for preparing an extract of a plant of the genus *Trichocaulon* or of the genus *Hoodia*, the extract comprising an appetite suppressant agent, the process including the steps of treating collected plant material with a solvent to extract a fraction having appetite suppressant activity, separating the extraction solution from the rest of the plant material, removing the solvent from the extraction solution and recovering the extract. The extract so recovered may be further purified, eg by way of suitable solvent extraction procedures.

The invention also provides a plant extract made of plants of the group comprising the genus *Trichocaulon* and the genus *Hoodia* and having appetite suppressant activity.

The extract may be prepared from plant material such as the stems and roots of said plants of the genus *Trichocaulon* or of the genus *Hoodia*. The genus *Trichocaulon* and the genus *Hoodia* include succulent plants growing in arid regions such as are found in Southern Africa. In one application of the invention, the active appetite suppressant extract is obtained from the species *Trichocaulon piliferum*. The species *Trichocaulon officinale* may also be used to provide an active appetite suppressant extract. In another application of the invention, the active appetite suppressant extract may be obtained from the species *Hoodia currorii*, *Hoodia gordonii* or *Hoodia lugardii*. Bioassays conducted by the Applicant on rats have indicated that certain of the extracts possess appetite suppressant activity.

The plant material may be homogenised in the presence of a suitable solvent, for example, a

methanol/methylene chloride solvent, by means of a device such as a Waring blender. The extraction solution may then be separated from the residual plant material by an appropriate separation procedure such as, for example, filtration or centrifugation. The solvent may be removed by means of the rotary evaporator, preferably in a water bath at a temperature of 60.degree. C. The separated crude extract may then be further extracted with methylene chloride and water before being separated into a methylene chloride extract and a water extract. The methylene chloride extract may have the solvent removed preferably by means of evaporation on a rotary evaporator and the resultant extract may be further purified by way of a methanol/hexane extraction. The methanol/hexane extraction product may then be separated to yield a methanol extract and a hexane extract. The methanol extract may be evaporated to remove the solvent in order to yield a partially purified active extract.

The partially purified active extract may be dissolved in methanol, and may be further fractionated by column chromatography, employing silica gel as an adsorption medium and a chloroform/30% methanol mixture as an eluent. A plurality of different fractions may be obtained, and each may be evaluated, by suitable bioassaying procedures, to determine the appetite suppressant activity thereof.

A fraction having appetite suppressant activity may preferably be further fractionated such as by column chromatography using silica gel as an adsorption medium and a 9:1 chloroform:methanol solvent, and the resultant sub-fractions bioassayed for their appetite suppressant activity. A sub-fraction displaying appetite suppressant activity may, if desired, be further fractionated and purified, conveniently using a column chromatographic procedure with silica gel as the adsorption medium and a 9:1 ethylacetate:hexane solvent. The resultant purified fractions may again be evaluated by suitable bioassay procedures for their appetite suppressant activity.

The Applicant has found that at least one such purified fraction has good appetite suppressant activity, and the active principle in the fraction was identified by conventional chemical techniques including nuclear magnetic resonance, and was found to be a compound of the structural formula ##STR2##

In accordance with S.I. nomenclature, the active principle (1) is the compound 3-O-[-.beta.-D-thevetopyranosyl-(1.fwdarw.4)-.beta.-D-cymaropyranosyl-(1.fwdarw.4)-.beta.-D-cymaropyranosyl]-12.beta.-O-tigloyloxy-14-hydroxy-14.beta.-pre gn-50-en-20-one (C.sub.47 H.sub.74 O.sub.15 M.sup.+ 878).

According to another aspect of the invention, there is provided a process for preparing an extract of a plant of the genus *Trichocaulon* or of the genus *Hoodia*, the extract comprising an appetite suppressant agent, the process including the steps of pressing collected plant material to separate sap from solid plant material and recovering the sap free of the solid plant material to form the extract.

The extract may be dried to remove moisture, e.g. by spray-drying, freeze-drying or vacuum drying, to form a free-flowing powder.

The invention extends to a composition having appetite suppressant activity comprising an extract as described above.

The composition may be admixed with a pharmaceutical excipient, diluent or carrier and optionally it is prepared in unit dosage form.

The invention also extends to the use of an extract as described above in the manufacture of a medicament having appetite suppressant activity, to an extract as described above for use as a medicament having appetite suppressant activity, and to a method of suppressing an appetite by administering to a human or animal an effective dosage of a composition as described above.

Compound (1) is a novel compound and the invention extends to compound (1) and certain analogues or derivatives of this steroidal trisaccharide having appetite suppressant properties. The molecules chosen as the analogues or derivatives are intended to affect the properties of the steroidal trisaccharide with the aim of increasing the activity of the active ingredient. The following effects were taken into consideration when the analogues were chosen:

(i) Hydrophobic interactions and lipophilicity

Functional group modifications of the active molecule is intended to change the hydrophobicity and lipophilicity of the molecule. Increased lipophilicity has been shown to correlate with increased biological activity, poorer aqueous solubility, increased detergency/cell lysis, increased storage in tissues, more rapid metabolism and elimination, increased plasma protein binding and faster rate of onset of action.

(ii) Electronic properties and ionization constants

Functional group modification of the molecule is also intended to change the acidity and basicity which would have a major role in controlling the transport of the compound to its site of action and the binding at this target site.

(iii) Hydrogen bonding

Functional group modifications of carboxyl and carbonyl groups in the active molecule are intended to change the interactions between the proteins in biological systems and the chemically modified functional groups.

(iv) Steric parameters

The purpose of changing the steric features of the molecule is to increase binding to its receptor and thus increase its biological activity.

The following chemical modifications to the molecule are intended to affect the hydrophobicity and lipophilicity electronic properties, hydrogen bonding and steric parameters on the molecule:

- a) Chemical modification of the C-12 group and ester functionality;
- b) Chemical modification of the 5,6-double bond, e.g. hydrogenation and migration;
- c) Chemical modification of the C-20 carbonyl and C-17 acetyl group;
- d) Chemical modification of the "D" ring of the steroid or aglycone ring;
- e) Modification of the carbohydrates of the trisaccharide moiety.

Accordingly, the invention provides a compound having the general structural formula ##STR3##

in which R=alkyl;

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5 or C5-C6.

The invention also provides a compound as described above wherein there is a further bond between C5-C6, R=methyl, R.sub.1 =tigloyl, R.sub.2 =3-0-[-.beta.-D-thevetopyranosyl-(1.fwdarw.4)-.beta.-D-cymaropyranosyl-(1.fwdarw.4)-.beta.-D-cymaropyranosyl] and having the structural formula. ##STR4##

Further active analogues or derivatives of the appetite suppressant compound (1) in accordance with the invention are compounds having the following structural formulae: ##STR5##

in which R=alkyl; and

R.sub.1.dbd.H or benzoyl, or, tigloyl, or any other organic ester group ##STR6##

in which R=alkyl; and

R.sub.1.dbd.H, or tigloyl, or benzoyl, or any other organic ester group ##STR7##

in which R=alkyl; and

R.sub.1.dbd.H, or tigloyl, or benzoyl, or any other organic ester group ##STR8##

in which R=alkyl; and

R.sub.1.dbd.H, or tigloyl, or benzoyl, or any other organic ester group ##STR9##

in which R=alkyl;

R.sub.1.dbd.H, or tigloyl, or benzoyl, or any other organic ester group. ##STR10##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5 or C5-C6. ##STR11##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the presence of a further bond between C4-C5 or C5-C6.

##STR12##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5 or C5-C6.

##STR13##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, or any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5, C5-C6 or C14-C15. ##STR14##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5, C5-C6 or C14-C15. ##STR15##

in which R=alkyl; and

R.sub.1.dbd.H, alkyl, tigloyl, benzoyl, any other organic ester group;

R.sub.2.dbd.H, or one or more 6-deoxy carbohydrates, or one or more 2,6-dideoxy carbohydrates, or glucose molecules, or combinations thereof;

and in which the broken lines indicate the optional presence of a further bond between C4-C5, C5-C6 or C14-C15; and

R.sub.3.dbd.H, alkyl, aryl, acyl, or glucoxy. ##STR16##

in which R.dbd.H, alkyl, aryl or any steroid possessing a C14 beta hydroxy group, or a C12 beta hydroxy functionality, or a C17 acyl group, or a C5-C6 olefin, or combinations thereof.

The invention still further extends to a process for synthetically producing a compound having appetite suppressant activity.

The process uses a steroid as a starting material (or intermediate or precursor), the steroid having the

chemical formula ##STR17##

The steroid (15) can be prepared from a compound having the formula (22) by a process which includes the steps of

(i) treating progesterone having the formula ##STR18##

with the micro-organism *Calonectria decora* to produce a compound 12.beta., 15.alpha.-dihydroxy progesterone of the formula ##STR19##

(ii) treating compound (17) with tosyl chloride and pyridine to produce a compound 12.beta.-hydroxy-15.alpha.-(p-toluene sulfonyl)-progesterone of the formula ##STR20##

(iii) treating the compound (18) with collidine at 150.degree. C. to produce a compound 12.beta.-hydroxy-.DELTA..sup.4 -progesterone of the formula ##STR21##

(iv) treating the compound (19) with acetyl chloride and acetic anhydride at 120.degree. C., to produce a compound 3,12.beta.-diacetoxyregna-3,5,14-trien-20-one of the formula ##STR22##

(v) treating the compound (20) with ethylene glycol and a catalytic amount of p-toluene sulphonic acid, to produce a compound 3,12.beta.-diacetoxy-20,20-ethylenedioxyregna-3,5,14-triene of the formula ##STR23##

(vi) treating the compound (21) with NaBH.sub.4 to produce a compound 3.beta., 12.beta.-dihydroxy-20,20-ethylenedioxyregna-5,14-diene-12-acetate of the formula ##STR24##

In a first alternative procedure, a process for the preparation of steroid (15) according to the invention includes the steps of

(a) treating compound (22) with a reducing agent, e.g. LiAlH.sub.4, to produce a compound 3.beta., 12.beta.-dihydroxy-20,20-ethylenedioxyregna-5,14-diene of the formula ##STR25##

(b) treating compound (23) with N-bromoacetamide (NBA) and a base, e.g. pyridine, to produce a compound 3.beta., 12,6,20-dihydroxy-14,15-epoxy-20,20-ethylenedioxyregna-5-ene of the formula ##STR26##

(c) treating compound (24) with a reducing agent, e.g.

LiAlH.sub.4, e.g. with refluxing, to produce a compound 3.beta., 12.beta., 14.beta.-trihydroxy-20,20-ethylenedioxyregna-5-ene of the formula ##STR27##

and (d) treating compound (25) with an acid, e.g. acetic acid, and water to produce the steroid intermediate compound 3.beta., 12.beta., 14.beta.-trihydroxyregna-5-ene (15).

Reaction Scheme A depicts the procedure for the preparation of steroid intermediate (15) from compound (22) according to "the first alternative procedure" of the invention (and includes the preparation of compound (22) from compound (16) for illustrative purposes). ##STR28## ##STR29## ##STR30##

In a second alternative procedure, a process for the preparation of steroid (15) according to the invention includes the steps of

(a) treating compound (22) (3.beta., 12.beta.-dihydroxy-20,20-ethylenedioxy-pregna-5,14-diene-12-acetate) with p-toluenesulfonyl chloride and a base, e.g. pyridine, to produce a compound 3.beta., 12.beta.-dihydroxy-20,20-ethylenedioxy-pregna-5,14-diene-3-tosyl-12-acetate of the formula ##STR31##

(b) treating compound (26) with potassium acetate in a solvent, e.g. acetone, to produce a compound 6.beta., 12.beta.-dihydroxy-20,20-ethylenedioxy-3,5.alpha.-cyclopropan-14-ene-12-acetate of the formula ##STR32##

(c) treating the compound (27) with a reducing agent, e.g.

LiAlH<sub>4</sub>, and e.g. tetrahydrofuran, to produce a compound 6.beta., 12.beta.-dihydroxy-20,20-ethylenedioxy-3,5.alpha.-cyclopropan-14-ene of the formula ##STR33##

(d) treating the compound (28) with N-bromoacetamide, optionally acetic acid, and a base, e.g. pyridine, to produce a compound 6.beta., 12.beta.-dihydroxy-20,20-ethylenedioxy-14,15-epoxy-3,5.alpha.-cyclopropan-14-ene of the formula ##STR34##

(e) treating the compound (29) with a reducing agent, e.g. LiAlH<sub>4</sub>, and e.g. tetrahydrofuran, to produce a compound 6.beta., 12.beta., 14.beta.-trihydroxy-20,20-ethylenedioxy-3,5.alpha.-cyclopropane of the formula ##STR35##

and (f) treating compound (30) with an acid, e.g. hydrochloric acid, and a solvent e.g. acetone, to produce compound (15).

Reaction Scheme B shows the procedure for the preparation of steroid intermediate (15) from compound (22) according to "the second alternative procedure" of the invention. ##STR36##

Compound (I) may be synthesized from a first carbohydrate intermediate in the form of an activated monosaccharide cymarose moiety, which can be prepared from a compound having the formula (36). Compound (36) can be prepared by a process which includes the steps

(i) treating methyl-.alpha.-D-glucose having the formula ##STR37##

with benzaldehyde and zinc chloride to produce a compound methyl-4,6-O-benzylidene-.alpha.-D-glucopyranoside of the formula ##STR38##

(ii) treating the compound (32) with tosyl chloride and pyridine at 0.degree. C., to produce a compound methyl-4,6-O-benzylidene-2-O-tosyl-.alpha.-D-glucopyranoside of the formula ##STR39##

(iii) treating the compound (33) with NaOMe at 100.degree. C. to produce a compound methyl 4,6-O-benzylidene-3-O-methyl-.alpha.-D-glucopyranoside of the formula ##STR40##

(iv) treating the compound (34) with N-bromosuccinamide (NBS) to produce a compound methyl 6-bromo-4-O-benzoyl-3-O-methyl-6-deoxy-.alpha.-D-glucopyranoside of the formula ##STR41##

and (v) treating the compound (35) with NaBH<sub>4</sub> and NiCl<sub>2</sub>, to produce a compound methyl 4-O-benzoyl-3-O-methyl-6-deoxy-.alpha.-D-glucopyranoside of the formula ##STR42##

The invention extends to a process for the preparation of a carbohydrate intermediate in the form of an

activated monosaccharide cymarose moiety which includes the steps of

(i) treating the compound (36) with  $\text{PhSSiMe}_3$ ,  $\text{ZnI}_2$  and  $\text{Bu}_4\text{I}^+ \text{I}^-$  to produce a compound 4-0-benzoyl-3-0-methyl-6-deoxy-.alpha..beta.-D-phenylthioaltroside of the formula ##STR43##

(ii) optionally treating the compound (37) with diethylaminosulphur trifluoride (DAST), e.g. at 0.degree. C., to produce a compound 4-0-benzoyl-3-0-methyl-2-phenylthio-2,6-dideoxy-.alpha..beta.-D-fluorocymaropyranoside having the formula ##STR44##

or (iii) optionally, treating the compound (37) with t-butyldimethylsilylchloride and imidazole in a solvent, e.g. pyridine, to produce 4-0-benzoyl-3-0-methyl-2-0-t-butyldimethylsilyl-.alpha..beta.-D-phenylthio altroside having the formula ##STR45##

in which Z=TBDMS=t-butyldimethylsilyl

and (iv) treating the compound (39) with a base, e.g. sodium methoxide, to produce 3-0-methyl-2-0-t-butyldimethylsilyl-.alpha..beta.-D-phenylthioaltroside having the formula ##STR46##

in which Z=TBDMS=t-butyldimethylsilyl.

Reaction Scheme C shows the procedure for the synthesis of the activated monosaccharide cymarose moiety (40) from compound (36) according to the invention (and includes the preparation of compound (36) from compound (31) for illustrative purposes). ##STR47##

The synthesis of compound (1) may also involve a second carbohydrate intermediate in the form of an activated monosaccharide thevetose moiety, which can be prepared from a compound having the formula (47). Compound (47) can be prepared by a process which includes the steps of

(i) treating .alpha.-D-glucose having the formula ##STR48##

with acetone and sulphuric acid to produce a compound 1,2:5,6-di-0-isopropylidene-.alpha.-D-glucofuranose of the formula ##STR49##

(ii) treating the compound (42) with NaH and MeI to produce a compound 1,2:5,6-Di-0-isopropylidene-3-0-methyl-.alpha.-D-glucofuranose of the formula ##STR50##

(iii) treating the compound (43) with acetic acid to produce a compound 3-0-methyl-.alpha..beta.-D-glucopyranose of the formula ##STR51##

(iv) treating the compound (44) with methanol and hydrochloric acid to produce a compound methyl 3-0-methyl-.alpha..beta.-D-glucopyranoside having the formula ##STR52##

(v) treating the compound (45) with benzaldehyde and zinc chloride to produce a compound methyl 4,6-0-benzylidene-3-0-methyl-.alpha..beta.-glucopyranoside having the formula ##STR53##

(vi) treating the compound (46) with N-bromosuccinamide, nickel chloride and sodium borohydride to produce a compound methyl 4-0-benzoyl-3-0-methyl-6-deoxy-.alpha..beta.-glucopyranoside having the formula ##STR54##

The invention extends to a process for the preparation of an activated monosaccharide thevetose moiety

which includes the steps of

(i) treating the compound (47) with phenylthiotrimethylsilane and trimethylsilyltrifluoromethanesulphonate to produce a compound 4-0-benzoyl-3-0-methyl-1-phenylthio-6-deoxy-.alpha..beta.-glucopyranoside having the formula ##STR55##

(ii) treating the compound (48) with pivaloyl chloride and a solvent, e.g. pyridine, to produce a compound 4-0-benzoyl-3-0-methyl-2-0-pivaloyl-1-phenylthio-6-deoxy-.alpha..beta.-glu copyranoside having the formula ##STR56##

and (iii) treating the compound (49) with a brominating agent, e.g. N-bromosuccinimide, and diethylaminosulphur trifluoride to produce a compound 4-0benzoyl-3-0-methyl-2-0-pivaloyl-1-stereo-isomers having the formula ##STR57##

Reaction Scheme D shows the procedure for the synthesis of the activated monosaccharide thevetose moiety (50(A) and 50(B)) from compound (48) according to the invention (and includes the preparation of compound (47) from compound (41) for illustrative purposes) ##STR58##

According to a still further aspect of the invention there is provided a process of synthetically producing a compound of the formula (1) and analogues and derivatives thereof which includes the steps of synthesising a suitable steroid intermediate or precursor and coupling the required number of suitable monosaccharides with the steroid intermediate.

The invention also provides a process of coupling a monosaccharide cymarose with the steroid intermediate, which includes the steps of

(i) reacting a cymarose moiety (38) with a steroid intermediate (15), e.g. at -15.degree. C., and in the presence of tin chloride, in a solvent, e.g. ether, to produce a compound 3-0-[4-0-benzoyl-2-phenylthio-.beta.-D-cymaropyranosyl]-12,14-.beta.-dihydroxy-pregn-5-ene-20-one of the formula ##STR59##

and (ii) treating the compound (51) with tiglic acid chloride in pyridine and thereafter with a base, e.g. NaOMe, to produce a compound 3-0-[-2-phenylthio-.beta.-D-cymaropyranosyl]-12.beta.-tigloyloxy-14-hydroxy-14.beta.-pregn-5-ene-20-one of the formula ##STR60##

The invention extends to a process which includes coupling a monosaccharide cymarose moiety to a monosaccharide thevetose moiety and coupling the resultant disaccharide with the combined steroid product (52) to form compound (1).

The process of coupling the monosaccharide cymarose moiety to the monosaccharide thevetose moiety and coupling the resultant disaccharide to the combined steroid product (52) may include the steps of (4) coupling a selectively protected cymarose moiety (40) and a selectively protected thevetose moiety (50 A) using tin chloride (SnCl.sub.2) and silver trifluoromethanesulphonate, e.g. at -15.degree. C., to produce a compound of the formula ##STR61##

in which Z=TBDMS=t-butyl dimethylsilyl

(ii) treating compound (53) with tetrabutylammonium fluoride to produce a compound of the formula ##STR62##

(iii) treating compound (54) with diethylaminosulphur trifluoride, e.g. at 0.degree. C., to produce a compound of the formula ##STR63##

(iv) reacting compound (55) with compound (52) to produce a compound of the formula ##STR64##

and (v) treating compound (56) in a Raney-Nickel reaction and thereafter with a base, e.g. NaOMe, to produce compound (1) as described above.

Reaction Scheme E shows the procedure for the synthesis of intermediates (52) and (55) and coupling them to form compound (56). ##STR65## ##STR66##

According to the invention, an alternative process is provided which includes coupling cymarose and thevetose moieties to form a trisaccharide and coupling the trisaccharide onto a steroid derivative to form a compound of the formula (1).

The process of forming the trisaccharide and coupling the resultant trisaccharide to a steroid derivative may include the steps of

(i) coupling a selectively protected cymarose moiety (40) and compound (45) using tin (II) chloride, AgOTf, Cp.sub.2 ZrCl.sub.2 to produce a compound of the formula ##STR67##

in which Z=TBDMS=t-butyl dimethylsilyl

(i) treating compound (57) with tetrabutylammonium fluoride and diethylaminosulphur trifluoride to produce a trisaccharide compound having the formula ##STR68##

and (iii) coupling the trisaccharide (58) with a steroid intermediate of the formula ##STR69##

using tin (II) chloride, AgOTf, Cp.sub.2 ZrCl.sub.2 to produce compound (1).

The steroid intermediate (59) may be produced by treating steroid (15) with tiglic acid chloride.

Reaction Scheme F shows the procedure for the synthesis of the trisaccharide (58) and the synthesis of compound (1) by coupling the trisaccharide (58) with the steroid intermediate (59). ##STR70##

The intermediates (23), (24), (25), (27), (28), (29), (30), (37), (38), (39), (40), (48), (49), (50), (51), (53), (54), (55), (56), (57) and (58) described above are novel compounds and the invention extends to these compounds as such.

Compound (1), 3-O-[-.beta.-D-thevecopyranosyl-(1.fwdarw.4)-.beta.-D-cymaropyranosyl-(1.fwdarw.4)-.beta.-D-cymaropyranosyl]-12.beta.-O-tigloyloxy-14-hydroxy-14.beta.-pre gn-5-en-20-one, and various analogues and derivatives thereof have been found to have appetite suppressing activity.

The invention extends also to a composition or formulation having appetite suppressant activity, in which the active ingredient is an extract obtained from a plant of the genus *Trichocaulon* or the genus *Hoodia*.

The active ingredient may be a compound of the formula (1), extracted from a plant of the genus *Trichocaulon* or *Hoodia* or a derivative thereof. The plant may be of the species *Trichocaulon officinale* or *Trichocaulon piliferum*, or the species *Hoodia currorii*, *Hoodia gordonii* or *Hoodia lugardii*.

The invention extends also to a composition or formulation having appetite suppressant activity, in which the active ingredient is a synthetically produced compound of the formula (1) or a derivative or

analogue thereof, as hereinbefore set out with reference to compounds (2) to (14).

According to another aspect of the invention there is provided a method of suppressing an appetite by administering to a human or animal a suitable dosage of an appetite suppressant agent comprising an extract of a plant of the genus *Trichocaulon* or *Hoodia*. The extract may be incorporated in a composition or formulation including also pharmaceutically acceptable other ingredients.

The appetite suppressant agent may be an isolated natural chemical or a synthetic chemical compound of the formula: ##STR71##

or derivatives or analogues thereof, as set out before.

The appetite suppressant composition or formulation may consist of the appetite suppressant agent admixed with a pharmaceutical excipient, diluent or carrier. Other suitable additives, including a stabilizer and such other ingredients as may be desired may be added.

The invention extends to the use of compound (1) or its derivatives or analogues in the manufacture of a medicament having appetite suppressant activity.

The invention further extends to compound (1), or its derivatives or analogues as set out before, for use as a medicament having appetite suppressant activity.

A method of suppressing an appetite by administering to a human or animal an effective dosage of a composition as described above is also provided.

A method has been described herein for extracting a steroidal glycoside having appetite suppressant activity from plant material obtained from a plant of the *Trichocaulon* or *Hoodia* genus. The invention thus extends to an extract obtained from plant material of the *Trichocaulon* or *Hoodia* genus and containing a substantially pure steroidal glycoside of formula (1).

The invention extends also to a foodstuff or a beverage containing an effective quantity of the steroidal glycoside of the formula (1), or its derivatives or analogues as set out before, to have an appetite suppressant effect when ingested.

Molecular genetic studies have led to a considerable increase in the understanding of the regulation of appetite, satiety and bodyweight. These studies have revealed numerous central regulatory pathways, mediated by a number of neuropeptides. The maintenance of a normal body weight is achieved by an intricate balance between energy intake, food consumption, and energy expenditure. Energy homeostasis is subject to a wide range of influences, ultimately controlled by the brain. The different signals include such things as sense of smell and taste and gastro-intestinal signals such as distension of the gastro-intestinal tract, chemical signals to the gastric mucosa and blood-borne metabolites such as fatty acids and glucose.

Centrally, neuropeptide "Y" (NPY) which is negatively regulated by leptin, has been established as one of the positive regulators of feeding behaviour. Expression of the endogenous antagonist for melanocortin receptors has also been shown to be the basis for obesity in a particular model (the ob/ob mouse). Indeed deficiency at the MC4 melanocortin receptor completely replicates the obesity syndrome. Other mediators which have been shown to have roles in the energy balance include bombesin, galanin and glucagon-like peptide-1.

Without being bound by theory, the Applicant believes that compound (1) and its analogues as described



## FIG. 2.

It will be evident from FIG. 2 that the test group of rats dosed with the extract on day 5 displayed a substantially diminished food intake over the next two days, while a control group did not disclose a comparable reduced food intake. The food intake of the test group returned to normal, and in fact increased, from day 8 onwards.

## EXAMPLE 3

A preferred embodiment of a process in accordance with the invention for producing an extract having appetite suppressant activity is illustrated schematically by way of example in FIGS. 3 and 4, which two Figures together illustrate the comprehensive process. However, various other procedures may be used, as will be understood by persons skilled in the art.

Referring to FIG. 3, plant material of the genus *Trichocaulon* or the genus *Hoodia* is fed into a blender 3, eg a Waring blender, by way of feedline 1, with a solvent in the form of a methylene chloride/methanol solution introduced via feedline 2. The homogenised product is fed via line 4 into a separation stage 5, eg in the form of a filter or centrifuge, and the residual plant material is removed via line 27.

The solvent/extract mixture is fed via line 6 into an evaporation stage 7, where the solvent is removed, for example by means of a rotor evaporator. The dried crude extract is fed via line 8 into a further extraction stage 9 with the addition of a methylene chloride/water solution introduced via feedline 29 for further extraction, and then to a separation stage 13 by way of line 11, where the water fraction is removed via line 31. The dissolved extract fraction is fed via line 15 into a drier stage 17 where the solvent is evaporated, for example by a rotor evaporator.

Referring to FIG. 4, the dried extract is fed via line 10 into an extraction stage 12. A methanol/hexane solution is also fed via line 14 into the extraction stage 12 for further purification and extraction of the dried extract. The extract/methanol/hexane mixture is fed via line 16 into a separation stage 18, the hexane fraction is removed via line 20, and the methanol/extract mixture is then fed via line 22 into a drying stage 24. In the drying stage 24, the solvent is removed, eg by evaporation on a rotor evaporator.

The dried, partially purified active extract is fed via line 26 and with the addition of methanol via line 28 into a solution stage 30, and the dissolved fraction is fed via line 36 to a chromatography column 38.

In the column 38 the methanol soluble fraction is further fractionated, using silica gel and a chloroform/30% methanol solvent, into different fractions schematically indicated as fractions I to V. According to an actual fractionation procedure carried out by the Applicant, the fractionation procedure yielded the following fraction weights: I(3.9 g); II(2.6 g); III(2.1 g); IV(1.1 g) and V(2.0 g). These fractions are individually evaluated by a suitable bioassaying procedure (in a step not shown) and those fractions identified as fractions I and II, displaying marked appetite suppressant activity, are fed by feedlines 40 and 42 into columns 44 and 46 respectively where they are further fractionated and purified by column chromatography, again by using silica gel and a 9:1 chloroform:methanol system.

The sub-fractions II(A)-(C) obtained from column 44 do not, when assayed, display a noteworthy appetite suppressant activity, and may be recycled for further chromatography.

The sub-fractions I(A)-(L) obtained from column 46 are also evaluated (by an assaying step not shown), and the sub-fraction I(C) is found to have marked appetite suppressant activity.









## EXAMPLE 11

## 3.beta., 12.beta.-Dihydroxy-14,15-epoxy-20,20-ethylenedioxy-5-ene (24)

The mixture of 14,15- and 5,6-epoxides (14,4 g, 37,0 mmol) in dry tetrahydrofuran (200 ml) is added to a suspension of lithium aluminium hydride (1,69 g, 44,4 mmol) in dry tetrahydrofuran (300 ml). The reaction mixture is stirred at room temperature for 24 hours, after which it is worked up as described earlier by the addition of water (1,69 ml), and sodium hydroxide (15% soln, 1,69 ml). After filtration and evaporation of the solvent, the crude product is purified by silica gel column chromatography using methanol/chloroform (1:9) as solvent to give the unreacted 14,15 epoxy-20,20-ethylenedioxy-5-ene (24) is (300 mg, 2,1%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>): 5,31 (1H, m, H-6), 3,82-3,98 (4H, m, ethylene dioxy), 3,43-3,52 (1H, m, H-3), 3,41 (1H, s, H-15), 3,31-3,35 (1H, dd, J=4,3 Hz, H-12), 1,29 (3H, s, H-21), 1,17 (3H, s, H-19), 1,02 (3H, s, H-18). <sup>13</sup>C NMR (CDCl<sub>3</sub>): 139,8 (C-5), 120,8 (C-6), 112,1 (C-20), 77,2 (C-12), 75,4 (C-14), 61,0 (C-15), 22,3 (C-21), 19,2 (C-19), 9,5 (C-18).

## EXAMPLE 12

## 3.beta., 12.beta., 14.beta.-Trihydroxy-20,20-ethylenedioxy-5-ene (25)

The 14,15-epoxide (24) (300 mg, 0,77 mmol) in dry tetrahydrofuran (10 ml) is added to a suspension of lithium aluminium hydride (300 mg, 7,89 mmol) in tetrahydrofuran and the reaction refluxed for 48 h. After the addition of water (0,3 ml), sodium hydroxide (15% soln, 0,3 ml) and filtration as described earlier, the mixture is purified by silica gel column chromatography using methanol: chloroform (1:9) as solvent to give the trihydroxy pregnene (25) (250 mg, 83%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>): 5,38 (1H, m, H-6), 3,98 (4H, m, ethylene dioxy), 3,43-3,53 (1H, m, H-3), 3,25-3,32 (1H, dd, J=4,1 Hz, H-12), 1,32 (3H, s, H-21), 1,01 (3H, s, H-19), 0,98 (3H, s, H-18). <sup>13</sup>C NMR CDCl<sub>3</sub>: 139,1 (C-5), 122,1 (C-6), 112,2 (C-20), 85,1 (C-14), 75,1 (C-12), 71,6 (C-3), 23,4 (C-21), 19,4 (C-19), 8,9 (C-18).

## EXAMPLE 13

## 3.beta., 12.beta., 14.beta.-Trihydroxy-pregn-5-ene (15)

The ethylenedioxy-5-ene (25) (250 mg, 0,64 mmol) is dissolved in acetic acid (13,4 ml) and water which after freeze drying affords the trihydroxy steroid (15) (200 mg, 89%), m.p.: 228.degree.-235.degree. C. (lit 225.degree.-235.degree. C.), M<sup>+</sup> 348, [ $\alpha$ ]<sub>D</sub><sup>20</sup> +35.degree. (lit [ $\alpha$ ]<sub>D</sub><sup>20</sup> +29.degree.).

<sup>1</sup>H NMR (CDCl<sub>3</sub>): 5,39 (1H, m, H-6), 3,56-3,62 (1H, t, J=8,1 Hz, H-17), 3,42-3,51 (1H, m, H-3), 3,28-3,39 (1H, dd, J=4,3 Hz, H-12), 2,23 (3H, s, H-21), 1,01 (3H, s, H-19), 0,90 (3H, s, H-18). <sup>13</sup>C NMR (CDCl<sub>3</sub>): 217,7 (C-20), 138,9 (C-5), 122,2 (C-6), 85,5 (C-14), 73,6 (C-12), 71,6 (C-3), 57,0 (C-17), 55,1 (C-13), 43,6 (C-9), 42,1 (C-4), 37,3 (C-1), 36,8 (C-10), 35,9 (C-8), 34,5 (C-15), 32,9 (C-21), 31,5 (C-16), 30,1 (C-2), 27,4 (C-7), 24,4 (C-11), 19,4 (C-19), 8,3 (C-18)

Examples 14 to 19 illustrate the synthetic procedures whereby the intermediate compounds and steroid (15) may be prepared according to "the second alternative procedure".

## EXAMPLE 14

## 20,20-Ethylenedioxy-3.beta.-toluene-p-sulphonyloxy-pregn-5,14-diene-12.beta.-ol acetate (26)

A solution of p-toluenesulphonyl chloride (650 mg, 3.4 mmol) in pyridine (10 ml) was added dropwise to a mixture of the 20,20-Ethylenedioxy-pregna-5,14-diene-3.beta.,12.beta.-diol 12-acetate (22) (1.3 g, 3.1 mmol) in pyridine (15 ml) at 0.degree. C. The reaction mixture was left stirring at room temperature for 24 hours after which water was added to the reaction mixture. The solution was extracted with ethyl acetate (2.times.50 ml), the ethyl acetate layer was washed citric acid (5.times.50 ml), saturated sodium bicarbonate solution (100 ml), saturated sodium chloride solution (100 ml) and water (100 ml) The ethyl acetate was dried (MgSO.sub.4), filtered, and evaporated and purified by flash column chromatography using hexane-ethyl acetate (8:2 v/v) as the eluant to give the .beta.-O-tosyl steroid (26), (1.5 g, 84%), as a yellow oil, (Found M 570.271, C.sub.32 H.sub.42 O.sub.7 S requires: M 570.273).

.delta..sub.H 1.021 (3H, s, 19-H), 1.131 (3H, s, 18-H), 1.282 (3H, s, 21-H), 2.021 (acetateOCH.sub.3), 2.431 (3H, s, Ar-CH.sub.3), 3.883 (4H, m, OCH.sub.2 CH.sub.2 O), 4.750 (1H, dd, .sup.3 J 10.8 Hz, 5.2 Hz, 12-H), 4.890 (1H, m, 30H), 5.281 (1H, dd, .sup.3 J 4.2 Hz, 2.1 Hz, 15-H), 5.388 (1H, m, 6-H), 7.341 (2H, d, .sup.3 J 8.2 Hz, ArH), 7.746 (2H, d, .sup.3 J 8.2 Hz, ArH). .delta..sub.C 13.493Q (C-18), 19.002Q (C-19), 21.612Q (Ar-methyl)\*, 21.671Q (C-21)\*, 24.175Q (acetate methyl), 63.401T (ethylenedioxy), 63.498T (ethylenedioxy), 71.531S (C-13), 80.912D (C-12), 82.531D (C-3), 111.363S (C-20), 120.881D (C-15), 121.461D (C-6), 123.715-133.917 (Aromatic), 139.903S (C-14), 151.722S (C-5), 170.819S (ester carbonyl).

\*may be interchanged

## EXAMPLE 15

## 20,20-Ethylenedioxy-3.alpha., 5-cyclo-5.alpha.-pregn-14-ene-6.beta., 12.beta.-diol-12-acetate (27)

A solution of 3.beta.-toluene-p-sulphonyloxy-pregn-5,14-diene (26) (1.2 g, 2.1 mmol) and potassium acetate (2.2 g, 22.4 mmol) in water (250 ml) and acetone (500 ml) was refluxed at 60.degree. C. for 16 hours. The acetone was evaporated and the water was extracted with ethyl acetate (200 ml). The ethyl acetate was dried (MgSO.sub.4), filtered, and evaporated. Flash chromatographic separation of the mixture using chloroform-acetone (9:1 v/v) as the eluant gave the 3.alpha.,5-cyclo derivative (27), (530 mg, 61%) as a yellow oil, (Found M 416.262, C.sub.25 H.sub.36 O.sub.5 requires: M 416.263).

.delta..sub.H 0.288 (1H, dd, .sup.3 J 8.1 Hz, 4.9 Hz, 4H.sub.a), 0.477 (1H, dd, .sup.3 J 4.4 Hz, 4.4 Hz, 4H.sub.b), 1.025 (3H, s, 19-H), 1.121 (3H, s, 18-H), 1.256 (3H, s, 21-H), 1.989 (3H, s, acetate-CH.sub.3), 3.302 (1H, dd, .sup.3 J 2.8 Hz 2.8 Hz, 6-H), 3.784-3.947 (4H, m, OCH.sub.2 CH.sub.2 O), 4.721 (1H, dd, .sup.3 J 8.5 Hz, 5.6 Hz, 12-H), 5.232 (1H, dd, .sup.3 J 3.9 Hz, 1.9 Hz, 15-H). .delta..sub.C 11.678T (C-4), 12.298Q(C-18), 19.971Q (C-19), 23.623Q(C-21), 24.153Q (acetate methyl), 63.700T (ethylenedioxy), 63.788T (ethylenedioxy), 73.591D (C-6), 80.551D (C-12), 111.126S (C-20), 118.778D (C-15), 152.959S (C-14), 170.991S (ester carbonyl).

## EXAMPLE 16

## 20,20-Ethylenedioxy-3.alpha., 5-cyclo-5.alpha.-pregn-14-ene-6.beta., 12.beta.-diol (28)

A solution of the 3.beta.,5-cyclo derivative (27), (500 mg, 1.2 mmol) in tetrahydrofuran (20 ml) was added dropwise to a suspension of lithium aluminium hydride (50 mg, 1.3 mmol) in tetrahydrofuran (10 ml). The reaction mixture was stirred for 4 hours and quenched by the addition of water (50 .mu.l). After

30 minutes, sodium hydroxide was added (15% solution, 50 .mu.l) and stirring continued for a further 30 minutes. Water (150 .mu.l) was added and the reaction mixture was filtered. The tetrahydrofuran was dried (MgSO<sub>4</sub>) filtered and evaporated and flash chromatographic purification using chloroform-acetone (8:2 v/v) as the eluant to give the diol (28), (370 mg, 83%) as an oil, (Found M 374.250, C<sub>23</sub>H<sub>34</sub>O<sub>4</sub> requires: M 374.252)

$\delta$ .sub.H 0.298 (1H, dd, .sup.3 J 8.1 Hz, 4.9 Hz, 4-H.sub.2), 0.510 (1H, dd, .sup.3 J 4.4 Hz, 4.4 Hz, 4-Hb), 0.985 (3H, s, 19-H), 1.055 (3H, s, 18-H), 1.325 (3H, s, 21-H), 3.318 (1H, dd, .sup.3 J 3.0 Hz, 3.0 Hz, 6-H), 3.363 (1H, dd, 3 J 11.4 Hz, 4.2 Hz, 12-H), 4.019 (4H, m, OCH.sub.2 Ch.sub.2 O) 4.622 (1H, s, OH), 5.255 (1H, dd, .sup.3 J 3.9 Hz, 1.9 Hz, 15-H).  $\delta$ .sub.C 11.681T(C-4), 12.243Q(C-18), 19.844Q (C-19), 23.604Q(C-21), 63.620T (ethylenedioxy), 63.733T (ethylenedioxy), 73.569D (C-6), 77.478D (C-12), 111.125S (C-20), 118.702D (C-15), 152.912S (C-14).

#### EXAMPLE 17

20, 20-Ethylenedioxy-14, 15.beta.-epoxy-3.alpha.,5-cyclo-5.alpha., 14.beta.-pregnane-6.beta.,12.beta.-diol (29)

N-bromoacetamide (150 mg, 1.1 mmol) was added to a solution of the 20,20-ethylenedioxy-3.alpha.,5-cyclo-5.alpha.-pregn-14-ene-6.beta.,12.beta.-diol (28) (340 mg, 0.91 mmol) in acetone (20 ml), water (0.25 ml) and acetic acid (0.25 ml) at 0.degree. C. After 15 min., sodium sulphite (5% solution, 20 ml) was added to the reaction mixture. The acetone was evaporated under reduced pressure and the remaining solution was extracted with dichloromethane (3.times.30 ml). The dichloromethane layer was dried (MgSO<sub>4</sub>), filtered and evaporated to a concentrated volume (50 ml). Pyridine (0.5 ml) was added to the mixture and stirred for a further 1 hour after which the dichloromethane layer was washed with a citric acid solution (5%, 3.times.30 ml), saturated sodium bicarbonate solution (30 ml) and water (30 ml). The dichloromethane layer was dried (MgSO<sub>4</sub>), filtered and evaporated and purified by flash column chromatography using chloroform-methanol (9.5:0.5 v/v) as the eluant to give the epoxide (29) (180 mg, 51% as a foam, (Found M 390.245, C<sub>23</sub>H<sub>34</sub>O<sub>5</sub> requires: M 390.247).

$\delta$ .sub.H 0.287 (1H, dd, .sup.3 J 8.1 Hz, 4.9 Hz, 4H.sub.a), 0.501 (1H, dd, .sup.3 J 4.4 Hz, 4.4 Hz, 4-H.sub.b), 0.978 (3H, s, 19-H), 1.048 (3H, s, 18-H), 1.321 (3H, s, 21-H), 3.318 (1H, dd, .sup.3 J 3.1 Hz, 3.1 Hz, 6-H), 3.355 (1H, dd, .sup.3 J 11.2 Hz, 4.1 Hz, 12-H), 3.491 (1H, s, 15-H), 4.001 (4H, m, OCH.sub.2 Ch.sub.2 O), 4.901 (1H, s, OH).  $\delta$ .sub.C 11.668T(C-4), 11.973Q(C-18), 19.515Q (C-19), 23.519Q(C-21), 59.910D (C-15), 63.601T (ethylenedioxy), 63.713T (ethylenedioxy), 72.501S (C-14), 73.571D (C-6), 77.471D (C-12), 111.085S (C-20).

#### EXAMPLE 18

20,20-Ethylenedioxy-6.beta.,12.beta., 14-trihydroxy-3.alpha.,5-cyclo-5.alpha., 14.beta.-pregnane (30)

A solution of the epoxide (29) (170 mg, 0.44 mmol) in tetrahydrofuran (10 ml) was added to a suspension of lithium aluminium hydride (20 mg, 0.53 mmol) in tetrahydrofuran (5 ml). The reaction mixture was refluxed for 2 hours after which water (20 .mu.l) was added and stirring continued for 0.5 hour. Sodium hydroxide solution (15%, 20 .mu.l) was added and stirring continued for a further 0.5 hour. A further quantity of water was added (60 .mu.l) and the suspension was stirred for 1 hour. After filtration, the suspension was dried (MgSO<sub>4</sub>) filtered, and the tetrahydrofuran was evaporated. Flash chromatographic separation of the resulting mixture eluting with chloroform-methanol (9:1 v/v) gave the required triol (30), 90 mg, 53%) as a clear oil, (Found M 392.261, C<sub>23</sub>H<sub>38</sub>O<sub>5</sub> requires: M 392.263).



## EXAMPLE 21

## Methyl-4,6-0-benzylidene-2-0-tosyl-.alpha.-D-glucopyranoside (33)

p-Toluene sulfonyl chloride (25 g, 1.2 eq) in pyridine (100 ml) is added dropwise to a solution of the benzylidene glucose (32) (31 g, 0.12 mol) in pyridine (100 ml) at 0.degree. C. The reaction is stirred at room temperature for 48 hours. Ice is added to the reaction mixture. The resulting white solid material is washed with water and recrystallized from hot ethanol to yield the tosylated glucose (33) (28 g, 60%).

## EXAMPLE 22

## Methyl-4,6-0-benzylidene-3-0-methyl-.alpha.-D-altropyranoside (34)

The tosylate (33) (28 g, 64 mmol) in a solution of sodium (7 g) in methanol (150 ml) is heated at 110.degree. C. for 48 hour in an autoclave. The reaction vessel is cooled and solid carbon dioxide is added to the reaction mixture. After filtration, the methanol is evaporated and the solid material is then taken up in water. The aqueous layer is extracted with chloroform (.times.3). The chloroform is dried (MgSO.sub.4), filtered and evaporated. The crude mixture is purified by silica gel column chromatography eluting with chloroform:acetone (9:1) to yield the altroside (34) (10 g, 52%)

## EXAMPLE 23

## Methyl-6-bromo-4-0-benzoyl-3-0-methyl-6-deoxy-.alpha.-D-altropyranoside (35)

The benzylidene altroside (34) (10 g, 33 mmol) is added to a solution of N-bromosuccinimide (7.6 g) and barium carbonate (20 g) in carbon tetrachloride and the reaction mixture is refluxed at 75.degree. C. for 3 hours. The reaction mixture is filtered and the carbon tetrachloride layer is washed with water. The organic layer is dried (MgSO<sub>4</sub>), filtered and evaporated to yield 6-bromo-altroside (35), (9 g, 69%).

## EXAMPLE 24

## Methyl-4-0-benzoyl-3-0-methyl-6-deoxy-.alpha.-D-altrolyranoside (36)

Sodium borohydride (18 g) in water (30 ml) is added dropwise to a solution of the bromoaltroside (35) (9 g, 23 mmol) and nickel chloride (18 g) in ethanol (300 ml) at 0.degree. C. The reaction mixture is refluxed at 75.degree. C. for 1 hour and then it is filtered. The ethanol is evaporated and the remaining aqueous layer is extracted with chloroform (.times.3). The chloroform is dried (MgSO.sub.4), filtered and evaporated, to yield the 6-deoxy-altroside (36) (5 g, 72%)

## EXAMPLE 25

## 4-0-Benzoyl-3-0-methyl-6-deoxy-.alpha..beta.-D-phenylthioaltropyranoside (37)

Phenylthiotrimethylsilane (5 ml) and trimethylsilyltrifluoromethane sulphonate (2 ml) are added at 0.degree. C. to a solution of the 6-deoxy-altroside (36) (5 g, 17 mmol) in dichloromethane (200 ml). The reaction mixture is stirred at room temperature for 6 hours. Saturated sodium bicarbonate is added to the reaction mixture. The dichloromethane layer is dried (MgSO.sub.4), filtered and evaporated. The crude mixture is purified by silica gel column chromatography eluting with chloroform:acetone (9:1) to yield the .alpha..beta.-phenylthioaltroside (37) (4 g, 63%)

## EXAMPLE 26

## 4-0-Benzoyl-3-0-methyl-2-phenylthio-2,6-dideoxy-.alpha..beta.-D-fluorocymar onyranoside (38)

Diethylaminosulphurtrifluoride (0,65 g) is added rapidly to a solution of the .alpha..beta.-phenylthioaltroside (37) (0,5 g, 1,33 mmol) in dichloromethane at 0.degree. C. The reaction is stirred for 0,5 h at 0.degree. C. and then saturated sodium bicarbonate is added. The dichloromethane is separated from the aqueous layer, dried (MgSO.sub.4), filtered and evaporated to yield the .alpha..beta.-fluorocymarose (38) (450 mg, 90%).

## EXAMPLE 27

## 4-0-Benzoyl-3-0-methyl-2-0-t-butyldimethylsilyl-.alpha..beta.-D-phenylthio- altroside (39)

The 6-deoxy altroside (37) (5 g) is silylated using t-butyldimethylsilylchloride (3 g) and imidazole (3 g) in pyridine (50 ml). The reaction is worked-up by extracting with ethyl acetate, washing the ethyl acetate with hydrochloric acid (6 N), then with sodium bicarbonate, and finally with water. The ethyl acetate layer is dried (MgSO.sub.4), filtered and evaporated to yield the silylated benzoyl phenylthioaltroside (39) (80%).

## EXAMPLE 28

## 3-0-methyl-2-0-t-butyldimethylsilyl-.alpha..beta.-D-phenylthioaltroside (40)

The silylated benzoyl phenylthioaltroside (39) (6 g) is treated with sodium methoxide (100 ml) for 4 hours. The methanol is evaporated and water is added to the reaction. The water layer is acidified (pH 5, ACOH) and extracted with ethyl acetate. The ethyl acetate is washed with water, dried (MgSO.sub.4), filtered and evaporated to yield silylated methyl phenylthioaltroside (40) (75%).

Examples 29 to 37 illustrate the procedures synthetic whereby the intermediate compounds may be prepared to form the second monosaccharide (50).

## EXAMPLE 29

## 1,2: 5,6-Di-O-isopropylidene-.alpha.-D-glucofuranose (42)

Sulfuric acid (40 ml) is added dropwise to a solution of .alpha.-D-glucose (41) (50 g, 0,28 mol) in acetone (1 l) at 0.degree. C. The reaction mixture is stirred for 24 h and then it is neutralized using sodium hydroxide (6 M). The acetone is evaporated and the aqueous layer is extracted with chloroform (X2). The chloroform is dried (MgSO.sub.4) filtered and evaporated. Crystallization from cyclohexane yielded the di-isopropylidene glucose (42) (41 g, 57%).

## EXAMPLE 30

## 1,2: 5,6-Di-O-isopropylidene-3-0-methyl-.alpha.-D-glucofuranose (43)

The .alpha.-D-glucofuranose (42) (41 g, 0,16 mol) in tetrahydrofuran (300 ml) is added dropwise to a suspension of sodium hydride (5 g) in tetrahydrofuran (200 ml). After 0,5 h, methyl iodide (25 g) in tetrahydrofuran (100 ml) is added dropwise to the reaction mixture which is then stirred for 24 h. Water is added to the reaction mixture which is then extracted with ether (.times.3). The ether layer is dried (MgSO.sub.4), filtered and evaporated to yield the methyl protected glucose (43) (38 g, 83%).

## EXAMPLE 31

## 3-0-Methyl-.alpha..beta.-D-glucopyranoside (44)

The methyl diisopropylidene compound (43) (38 g, 0,14 mol) is dissolved in acetic acid (50%, 700 ml) and the solution refluxed for 18 h. After cooling the acetic acid is evaporated. The crude product is purified by column chromatography eluting with chloroform:methanol:acetone:water (70:27:2 1) to yield 3-0-methyl-.alpha..beta.-glucopyranoside (44) (13 g, 50%).

## EXAMPLE 32

## Methyl 3-0-methyl-.alpha..beta.-D-glucopyranoside (45)

The 3-0-methyl-.alpha..beta.-glucopyranoside (44) (10 g) is dissolved in methanol (50 ml) and HCl (conc.) (1 ml) and refluxed overnight. Solid NaHCO<sub>3</sub> is added and the reaction is filtered. The methanol is evaporated to give 1,3-di-0-methyl-.alpha..beta.-D-glucopyranoside (45), (95%).

## EXAMPLE 33

## Methyl 4,6-0-benzylidene-3-0-methyl-.alpha..beta.-glucopyranoside (46)

The glucopyranoside (45) (8 g) is stirred at room temperature in a solution of benzaldehyde (20 ml) and zinc chloride (5 g). After 24 hours, ice is added and the aqueous layer is extracted with chloroform. The chloroform layer is dried (MgSO<sub>4</sub>), filtered and evaporated. The benzaldehyde is removed by vacuum distillation and the product is purified by silica gel column chromatography eluting with acetone:chloroform (0,5:9,5), to yield benzylidene-.alpha..beta.-glucopyranoside (46) (60%).

## EXAMPLE 34

## Methyl 4-0-benzoyl-0-methyl-6-deoxy-.alpha..beta.-glucopyranoside (47)

The benzylidene compound (46) (5 g) is refluxed at 80.degree. C. in a mixture of N-bromosuccinimide (3,7 g) and barium carbonate (4 g) in carbon tetrachloride. After 4 hours, the reaction is filtered and the carbon tetrachloride is washed with water, dried (MgSO<sub>4</sub>), filtered and evaporated to give the bromo compound (70%).

The bromo compound (4,3 g) is dissolved in a solution of ethanol (300 ml) and nickel chloride (8,6 g) at 0.degree. C. To this solution, sodium borohydride (8,6 g) in water (50 ml) is added dropwise over a period of 15 minutes. The reaction mixture is refluxed at 100.degree. C. for 45 minutes, cooled, filtered and evaporated. Chloroform is added, and the chloroform layer is washed with water, dried (MgSO<sub>4</sub>), filtered and evaporated to give the 6-deoxy sugar (47) (70%).

## EXAMPLE 35

## 4-0-Benzoyl-3-0-methyl-1-phenylthio-6-deoxy-.alpha..beta.-glucopyranoside (48)

The 6-deoxy glucopyranoside (47) (3 g) is dissolved in dichloromethane (50 ml). To this solution, phenylthiotrimethylsilane (2 g) and trimethylsilyltrifluoromethanesulphonate (0,2 ml) are added. The solution is stirred at room temperature overnight, after which saturated sodium bicarbonate is added. The dichloromethane layer is dried (MgSO<sub>4</sub>), filtered and evaporated. The product is purified by silica gel column chromatography eluting with ethyl acetate:hexane (2:8), to give the compound (48) (60%).



## Thevetose-cymarose dissaccharide (54)

To a solution of the dissaccharide (53) (200 mg) in tetrahydrofuran (20 ml), tetrabutylammonium fluoride (0,4 ml) is added. The mixture is stirred at room temperature for 1 hour, after which saturated sodium bicarbonate is added. The reaction mixture is extracted with ethyl acetate and the ethyl acetate layer is dried (MgSO.sub.4), filtered and evaporated. The dissaccharide (54) is purified by silica gel column chromatography (acetone:chloroform, 0,5:9,5) yield 60%.

## EXAMPLE 41

## Thevetose-cymarose dissaccharide (55)

To a solution of the dissaccharide (54) (80 mg) in dichloromethane (10 ml), diethylamino sulphur trifluoride (80 .mu.l) is added at 0.degree. C. After stirring at 0.degree. C. for 0,5 hour, saturated sodium bicarbonate and more dichloromethane are added. The dichloromethane is dried (MgSO.sub.4), filtered and evaporated. Purification by silica gel column chromatography (ethyl acetate:hexane 1:9), gives the dissaccharide (55) in a 65% yield.

## EXAMPLE 42

The results of the following three bioassays on the appetite suppressant are set out below, viz.

- a) Irwin Test;
- b) Acute Toxicity Test; and
- c) Oral Dose Anorectic Test.

## a) Irwin Test

The purpose of this test was to evaluate the appetite suppressant of the invention produced from a plant extract as hereinbefore described, according to the reduced animal Irwin test for tranquillising and sedative action.

## Experimental Procedure

The appetite suppressant was extracted from plant material by the Applicant by the method as hereinbefore described and administered to two of four groups of three animals each: one group receiving no treatment, one group receiving the solvent dimethylsulfoxide (DMSO), one group receiving the test sample at 50 mg/kg, and one group receiving the test sample at 300 mg/kg. Treatment took place by intraperitoneal injection, and observations were made at specific intervals up to five hours post treatment. Only symptoms other than those observed in the DMSO-treated animals were used in the interpretation of the results.

## Results

It was clear that the solvent, DMSO, had a marked effect on the animals, especially on the heat regulating mechanism. Body temperatures of all the animals treated with the solvent, alone or together with the test sample, showed a marked drop.

Animals in the low dose group showed decreased dispersion in the cage and decreased locomotor





appearance of the liver was classified as moderate in both animals

#### Group 6 (1 600 mg/kg)

None of the animals presented any clinical signs of toxicity during the duration of the experiment. No macroscopic pathology was observed at post-mortem examination, but moderate degenerative changes in the liver of animal 11 were observed at histopathological examination. Animal 12 showed moderate cloudy swelling and mild hydropic changes of the hepatocytes. Food and water intakes were normal, as was the increase in body mass over the experimental period.

#### Group 7 (3 028,5 mg/kg)

Only one animal was treated at this dose. This animal showed no signs of toxicity during the observation period, and no macroscopic pathology was observed. At histopathological examination, moderate cloudy swelling and hydropic degeneration of the hepatocytes was observed. The animal showed a loss of body mass over the observation period (-0,82 g), but food and water intakes were normal.

#### Discussion

Since a very small number of animals were used in each dose group, it is difficult to make any conclusions. The fact that only one animal died at a low dose rate, without showing any symptoms, might indicate that death was not related to the test sample, but due to stress during and/or after treatment. No animals in higher dose groups died or showed any signs of toxicity, which further supports this assumption.

The increased food intake observed in animal 8 could possibly be ascribed to excessive spillage of food as was reflected in the small increase in body mass. It should be kept in mind that all the animals in this experiment were only treated once, and that it is unlikely that an appetite suppressor will have a marked influence on either the food or water intakes, or body mass over a 14 day period, as was the case in this experiment.

From the histopathological examination of the liver samples, it was clear that the pathological changes were dose related, with animals receiving higher doses showing the extensive changes. The pathology observed was not metabolic of nature, but possibly test sample-induced. The changes were only degenerative and therefore reversible. No signs of irreversible hepatocellular changes were observed.

It can, therefore, be concluded that only one animal died at a lower dose (800 mg/kg), but that the death was possibly not test sample related. None of the other animals in any of the dose groups showed any signs of toxicity during the 14 day observation period after treatment, or died as result of the treatment. A single oral dose of the test sample induced reversible dose-related hepatocellular changes.

#### c) Oral Dose Anorectic Test

The purpose of this test was to determine the activity of a plant extract prepared in accordance with the invention, and the minimum effective dose, and at the same time investigate any possible side-effects such as respiratory suppression, as experienced in the Irwin Test (referred to above).

#### Experimental Procedure

Animals were allocated to treatment groups using randomisation tables. Each treatment group consisted of three animals, with 6 animals in the control group. The test sample was dosed to young female rats

with body weight 100-150 g at acclimatisation, for three consecutive days. Animals were identified by means of metallic ear tags and KMnO<sub>4</sub> skin markings for easy identification. Animals were housed individually in standard rodent polycarbonate cages, and water and powdered commercial rodent pellets were available ad libitum. Water and food intakes were measured and calculated for each day. In order to find the minimum effective dose of the test sample, five doses were tested. Treatment was by oral gavage, with the test sample suspended in potato starch.

The test substance was compound (1), a white granular powder prepared from an extract from plant material in accordance with the invention, and the measured quantity of the test sample was mixed with prepared potato starch and dosed. Mixing with potato starch took place immediately before dosing on each day. Before withdrawal of the dosing volume for each animal, the suspensions were mixed thoroughly using a Vortex.

A range of five doses was tested, with a control group receiving only the carrier substance. Doses were chosen on the basis of the effects observed in the aforescribed Irwin Test and were:

Group 1: 0,00 mg/kg (Control Group)

Group 2: 6,25 mg/kg

Group 3: 12,50 mg/kg

Group 4: 25,00 mg/kg

Group 5: 37,50 mg/kg

Group 6: 50,00 mg/kg

## Results

Treatment did not affect the health of the animals during the study period. Animals treated with the test sample in all dose groups, showed a significantly reduced mean body mass gain over the total study period, and animals in three of the five treatment groups actually lost body mass.

Mean food intakes for all the treatment groups were reduced over the study period. Animals in the higher dose groups showed an increased water consumption.

Respiratory rate in none of the animals in any dose group was significantly effected.

Animals in all dose groups presented with friable livers at post-mortem examination, but no macroscopic pathology was observed.

## Discussion

Data collected during the acclimatisation period confirmed that all animals included in the experiment were healthy and body mass gain was comparable between the animals.

The reduction, and in some animals even a loss, in body mass gain, in combination with the reduced food intake is strongly indicative of suppression of the appetite centre.

Reduced food intake and reduced body mass gain was experienced even with the lowest dose group



|                     |               |
|---------------------|---------------|
| Outlet temperature  | 70.degree. C. |
| Chamber temperature | 78.degree. C. |

The spray-dried powder obtained was a free flowing powder (22 g) with a moisture content of 6.9%.

The spray dried powder was analysed for active ingredient concentration using HPLC techniques. The concentration of the active was determined to be 13 g/kg of spray dried powder.

#### HPLC Analysis Method

|                  |                                      |
|------------------|--------------------------------------|
| Eluant           | Acetonitrile: water (7:3), isocratic |
| Column           | Reverse phase C-18                   |
| UV absorbance    | 225 nm                               |
| Flow rate        | 1 ml/min                             |
| Injection volume | 10 .mu.l                             |

#### Method

Spray-dried powder (10 mg) was dissolved in water (0.5 ml) and acetonitrile (0.5 ml) 10 .mu.l of this solution was injected into the HPLC and the concentration of the active compound (1) was determined using a standard curve which was prepared from the pure compound (1).

#### EXAMPLE 44

The results of a study designed to assess the possible anorectic effects of compound (1) in the rat are presented below. In the following, the samples tested are pure sap (Sample 1), spray-dried sap (Sample 2) and active moiety (Sample 3). Samples 1 and 2 are the sap and the spray-dried sap respectively, as described in Example 43 above. Sample 3 is solvent-extracted compound (1) of .gtoreq.95% purity.

Sample 1 to 3 were each administered as a single oral dose to male Wistar rats. Two additional control groups received vehicle (distilled water or DMSO). Orally administered fenfluramine (7.5 mg/kg) was included as a reference standard.

Sample 1 (pure sap) administered orally, produced dose-dependent reductions in food consumption which were statistically significant at doses of 1600 mg/kg and above when compared with vehicle-treated controls. Concomitant reductions in bodyweight (or growth rate) were also recorded. On the day of dosing, statistically significant increases in water consumption were recorded at 3 hours post-dose (6400 and 10000 mg/kg) and 6 hours post-dose (10000 mg/kg). Between 24 and 48 hours post-dose, statistically significant reductions in water consumption were recorded at doses of 3200 mg/kg and above.

Sample 2 (spray-dried sap) administered orally at 76 mg/kg also produced statistically significant reductions in food consumption and bodyweight when compared with vehicle-treated animals. No statistically significant effects on water consumption were recorded.

Sample 3 (active moiety) produced statistically significant reductions in food consumption at an oral dose of 5.0 mg/kg. No statistically significant effects on bodyweights were produced by the active moiety although examination of the data revealed a slight delay in growth when compared with vehicle-

treated control animals. No statistically significant effects on water consumption were recorded.

The reference standard, fenfluramine (7.5 mg/kg), produced statistically significant reductions in food consumption at 6 and 24 hours post-dose when compared with the relevant vehicle-treated control group. No statistically significant effects on water consumption or bodyweight were recorded.

No treatment-related effects on the livers were recorded.

| Identity           | TEST SUBSTANCE              |                            |                            |
|--------------------|-----------------------------|----------------------------|----------------------------|
|                    | Sample 1 (pure sap)         | Sample 2 (spray-dried sap) | Sample 3 (active moiety)   |
| Appearance         | Brown liquid                | Powder                     | White powder               |
| Storage conditions | -20 .degree. C. in the dark | in the dark                | 4.degree. C. in the dark   |
| Purity             | Pure sap                    | Pure spray-dried sap       | .gtoreq.95%                |
| Vehicle            | Distilled water             | Distilled water            | Dimethyl-sulphoxide (DMSO) |

### Experimental Procedure

Fifty-five male Wistar rats were used for the study.

Bodyweights, food consumption (food hopper weight) and water consumption (bottle weight) were recorded daily at the same time each day from the day of arrival until the termination of the study.

On Day 1, the rats received a single oral (gavage) dose according to the following table:

| Group | n | Oral treatment            | Dose (mg/kg) |
|-------|---|---------------------------|--------------|
| 1     | 5 | Vehicle (distilled water) | --           |
| 2     | 4 | Sample 1 (pure sap)       | 800          |
| 3     | 5 | Sample 1 (pure sap)       | 1600         |
| 4     | 5 | Sample 1 (pure sap)       | 3200         |
| 5     | 5 | Sample 1 (pure sap)       | 6400         |
| 6     | 5 | Sample 1 (pure sap)       | 10000        |
| 7     | 5 | Sample 2 spray-dried sap  | 38           |
| 8     | 5 | Sample 2 spray-dried sap  | 76           |
| 9     | 5 | Sample 3 (active moiety)  | 2.5          |
| 10    | 5 | Sample 3 (active moiety)  | 5.0          |
| 11    | 3 | Fenfluramine              | 7.5          |
| 12    | 3 | Vehicle (DMSO)            | --           |

Groups 1-8 were dosed using a constant dose volume of 10 ml/kg and groups 9-12 were dosed using a dose volume of 1 mg/kg.

Food water consumption were also measured at 1,3 and 6 hours after dosing on Day 1.

Following the measurements of Day 8, the animals were killed by carbon dioxide asphyxiation, and the

livers excised and placed in lost buffered formalin, prior to histology. Paraffin wax sections of each liver were taken at 4-5  $\mu\text{m}$  and stained with haematoxylin and eosin. Additional sections were cut on a cryostat at 12  $\mu\text{m}$  and stained for fat with Oil Red O (ORO).

#### Data Analysis

The post-dose food and water consumption measurements and bodyweights at each time-point for the P57-treated animals were compared with those for the relevant, similarly-treated vehicle control group using analysis of variance followed by Williams' test for comparisons with controls.

The data for the fenfluramine-treated animals was compared with that for the vehicle-treated control group using Student's t test.

#### Results

The results are summarised in the tables.

Sample 1 (pure sap) administered orally produced marked, dose-related reductions in daily food consumption. The duration and amplitude of these reductions in food consumption were dose-dependent. At 24 hours post-dose, Sample 1 (pure sap) produced statistically significant reductions in food consumption at doses of 1600 mg/kg and above when compared with vehicle-treated controls. The highest dose of Sample 1 (sap) (10000 mg/kg) produced statistically significant reductions in food consumption on a daily basis up to 5 days post-dose.

Sample 2 (spray-dried sap) and Sample 3 (active moiety) produced marked and statistically significant reductions in food consumption at oral doses of 76 and 5.0 mg/kg respectively. In both cases the effects lasted 48 hours post-dose.

The reference standard, fenfluramine (7.5 mg/kg, p.o.) produced statistically significant reductions in food consumption at 6 and 24 hours post-dose when compared with the relevant vehicle-treated control group (Group 12).

Sample 2 (spray-dried sap) and Sample 3 (active moiety) produced no marked, dose-related effects on water consumption. On the day of dosing, the pure sap produced statistically significant increases in water consumption at 3 hours post-dose (6400 and 10000 mg/kg) and 6 hours post-dose (10000 mg/kg). Two days after dosing however, statistically significant decreases in water consumption were recorded in animals receiving Sample 1 (sap) at 3200, 6400 and 10000 mg/kg. These reductions however, were not clearly dose-related and only occurred between 1 and 2 days post-dose. The biological significance of these effects therefore remains unclear.

Sample 1 (pure sap) produced dose-related, statistically significant effects on bodyweights when compared with the vehicle-treated control group (Group 1). When administered orally at doses of 3200 mg/kg and above, Sample 1 (pure sap) produced statistically significant reductions in bodyweight or decreased growth rates when compared with vehicle-treated animals. These effects were statistically significant from 48 hours post-dose until the end of the study.

Sample 2 (spray-dried sap) administered orally at 76 mg/kg also produced statistically significant reductions in growth of the animals when compared with the vehicle-treated control group (Group 1). These effects were statistically significant between Days 3 (48 hours post-dose) and 5 inclusive.

Although Sample 3 (active moiety) appeared to delay the growth of the animals at the highest dose (5.0

mg/kg) when compared with the relevant vehicle-treated control group (Group 12), this effect was not statistically significant.

Fenfluramine, (7.5 mg/kg) produced no marked or statistically significant effects on water consumption or bodyweights when compared with the vehicle-treated control group (Group 12).

No treatment-related effects on the livers were recorded.

TABLE 1

Effects of oral administration on food consumption in the rat (daily pre-dose data)

| Group | Oral treatment         | Dose (mg/kg) | Group mean food consumption (g $\pm$ sd) between Days: |                 |                 |                 |                 |
|-------|------------------------|--------------|--|-----------------|-----------------|-----------------|-----------------|
|       |                        |              | -4--3  | -3--2           | -2--1           | -6--5           | -5--4           |
| 1     | Vehicle (water)        | --           | 27.6 $\pm$ 3.67  | 28.3 $\pm$ 3.50 | 29.4 $\pm$ 2.66 | 27.8 $\pm$ 1.54 | 24.2 $\pm$ 1.83 |
| 2     | Sample 1 sap           | 800          | 27.7 $\pm$ 0.76  | 28.4 $\pm$ 1.51 | 30.1 $\pm$ 0.27 | 28.3 $\pm$ 1.43 | 24.9 $\pm$ 0.82 |
| 3     | Sample 1 sap           | 1600         | 27.4 $\pm$ 1.96  | 28.8 $\pm$ 0.61 | 29.5 $\pm$ 1.55 | 29.0 $\pm$ 1.39 | 25.0 $\pm$ 2.16 |
| 4     | Sample 1 sap           | 3200         | 26.0 $\pm$ 2.52  | 28.5 $\pm$ 2.29 | 27.6 $\pm$ 1.15 | 27.2 $\pm$ 2.33 | 25.1 $\pm$ 2.46 |
| 5     | Sample 1 sap           | 6400         | 27.3 $\pm$ 1.45  | 29.2 $\pm$ 1.09 | 30.3 $\pm$ 0.90 | 28.7 $\pm$ 1.64 | 25.3 $\pm$ 1.73 |
| 6     | Sample 1 sap           | 10000        | 26.0 $\pm$ 2.31  | 27.0 $\pm$ 3.50 | 28.7 $\pm$ 2.26 | 28.5 $\pm$ 2.38 | 23.7 $\pm$ 2.73 |
| 7     | Sample 2 spray-dried   | 38           | 24.5 $\pm$ 2.30  | 27.6 $\pm$ 1.61 | 28.5 $\pm$ 1.87 | 28.1 $\pm$ 1.24 | 23.9 $\pm$ 1.79 |
| 8     | Sample 2 spray-dried   | 76           | 27.1 $\pm$ 1.01  | 28.7 $\pm$ 1.99 | 28.9 $\pm$ 1.37 | 28.7 $\pm$ 0.91 | 26.5 $\pm$ 1.55 |
| 9     | Sample 3 active moiety | 2.5          | 3.12   | 29.0 $\pm$ 1.99 | 29.4 $\pm$ 1.76 | 28.8 $\pm$ 1.49 | 26.4 $\pm$ .    |
| 10    | Sample 3 active moiety | 5.0          | 1.86   | 28.1 $\pm$ 2.65 | 28.0 $\pm$ 2.65 | 28.3 $\pm$ 2.1  | 25.8 $\pm$ .    |
| 11    | Fenfluramine           | 7.5          | 27.0 $\pm$ 1.53  | 30.8 $\pm$ 0.54 | 29.7 $\pm$ 2.84 | 29.1 $\pm$ 0.66 | 25.3 $\pm$ 4.03 |
| 12    | Vehicle (DMSO)         | --           | 28.7 $\pm$ 1.99  | 28.1 $\pm$ 4.06 | 30.5 $\pm$ 2.54 | 27.9 $\pm$ 1.8  | 26.7 $\pm$ 2.11 |

Effects of oral administration on food consumption in the rat (daily post-dose data)

| Group | Oral treatment  | Dose (mg/kg) | Group mean food consumption (g $\pm$ sd) between Days: |                   |                  |                 |                 |                 |                 |              |        |
|-------|-----------------|--------------|--|-------------------|------------------|-----------------|-----------------|-----------------|-----------------|--------------|--------|
|       |                 |              | 4-5  | 5-6               | 6-7              | 7-8             | 2-3             | 3-4             |                 |              |        |
| 1     | Vehicle (water) | --           | 3.49   | 31.8 $\pm$ 3.49   | 30.7 $\pm$ 2.24  | 31.7 $\pm$ 3.03 | 29.5 $\pm$ 3.15 | 29.6 $\pm$ 2.84 | 30.6 $\pm$ .    |              |        |
| 2     | Sample 1 sap    | 800          | 1.15   | 30.9 $\pm$ 0.60   | 33.3 $\pm$ 1.69  | 32.7 $\pm$ 0.80 | 26.1 $\pm$ 0.98 | 29.3 $\pm$ 1.49 | 30.7 $\pm$ .    |              |        |
| 3     | Sample 1 sap    | 1600         | 2.54   | 30.9 $\pm$ 1.22   | 34.1 $\pm$ 1.36  | 33.7 $\pm$ 1.69 | 22.6** $\pm$ .  | 3.17            | 26.9 $\pm$ 2.06 | 30.9 $\pm$ . |        |
| 4     | Sample 1 sap    | 3200         | $\pm$ 1.77   | 28.0 $\pm$ 3.14   | 31.4 $\pm$ 2.82  | 32.3 $\pm$ 2.91 | 20.1** $\pm$ .  | 1.39            | 19.0** $\pm$ .  | 1.88         | 22.8** |
| 5     | Sample 1 sap    | 6400         | $\pm$ 0.97   | 22.4** $\pm$ 3.01 | 26.9 $\pm$ 2.81  | 31.0 $\pm$ 2.31 | 18.2** $\pm$ .  | 4.18            | 14.8** $\pm$ .  | 1.75         | 18.4** |
| 6     | Sample 1 sap    | 10000        | $\pm$ 3.15   | 19.7** $\pm$ 4.31 | 22.6* $\pm$ 5.70 | 30.1 $\pm$ 4.79 | 15.1** $\pm$ .  | 2.98            | 12.4** $\pm$ .  | 2.61         | 16.0** |
| 7     | Sample 2 spray- | 38           |  |                   | 25.6 $\pm$ 2.85  | 27.3 $\pm$ 0.95 |                 |                 |                 |              |        |

|      |                |         |      |                     |      |      |       |     |      |      |     |      |
|------|----------------|---------|------|---------------------|------|------|-------|-----|------|------|-----|------|
| 2.06 | 31.0           | +-.     | 2.13 | 31.8                | +-.  | 1.63 | 31.1  | +-. | 1.94 | 31.8 | +-. | 2.45 |
|      | dried          |         |      |                     |      |      |       |     |      |      |     |      |
| 8    | Sample 2       | spray-  | 76   | 24.2*               | +-.  | 3.25 | 25.2* | +-. | 3.24 | 29.9 | +-. |      |
| 1.85 | 30.2           | +-.     | 2.28 | 31.2                | +-.  | 2.26 | 32.3  | +-. | 1.44 | 33.1 | +-. | 0.61 |
|      | dried          |         |      |                     |      |      |       |     |      |      |     |      |
| 9    | Sample 3       | active  | 2.5  | 26.8                | +-.  | 3.33 | 29.1  | +-. | 3.43 | 31.7 | +-. |      |
| 3.08 | 34.0           | +-.     | 2.95 | 34.4                | +-.  | 4.32 | 33.1  | +-. | 4.11 | 34.8 | +-. | 3.71 |
|      | moiety         |         |      |                     |      |      |       |     |      |      |     |      |
| 10   | Sample 3       | active  | 5.0  | 22.1 <sup>sup</sup> | +-.  | 3.07 | 27.6  | +-. | 5.26 | 30.5 | +-. | 3.16 |
|      | .dagger        | .dagger |      |                     |      |      |       |     |      |      |     |      |
| 32.4 | +-.            | 3.25    | 33.0 | +-.                 | 3.84 |      |       |     |      |      |     |      |
|      | moiety         |         |      |                     |      |      |       |     |      |      |     |      |
| 11   | Fenfluramine   |         | 7.5  | 22.4 <sup>sup</sup> | +-.  | 3.19 | 31.9  | +-. |      |      |     |      |
| 0.84 | 32.7           | +-.     | 2.50 | 33.0                | +-.  | 2.55 | 30.4  | +-. | 0.23 | 32.7 | +-. | 1.90 |
| 1.60 |                |         |      |                     |      |      |       |     |      |      |     |      |
| 12   | Vehicle (DMSO) |         | --   | 29.9                | +-.  | 3.36 | 30.6  | +-. | 4.43 | 30.1 | +-. |      |
| 4.17 | 32.4           | +-.     | 5.26 | 31.8                | +-.  | 3.08 | 32.8  | +-. | 3.98 | 33.3 | +-. | 3.76 |

sd Standard deviation  
 Groups 2-8 were compared with vehicle Group 1: \*p < 0.05, \*\*p < 0.01  
 Groups 9-11 were compared with vehicle Group 12: <sup>sup</sup>.dagger.p < 0.05,  
 !<sup>sup</sup>.dagger.dagger.p < 0.01

TABLE 2a  
 Effects of oral administration on water consumption in the rat (daily pre-dose data)

|                                     |                        | Group mean water |               |              |  |
|-------------------------------------|------------------------|------------------|---------------|--------------|--|
| consumption (g +- sd) between Days: |                        |                  |               |              |  |
| Group                               | Oral treatment         | Dose (mg/kg)     | -6--5         | -5--4        |  |
| -4--3                               | -3--2                  | -2--1            |               |              |  |
| 1                                   | Vehicle (water)        | --               | 40.9 +- 4.61  | 34.8 +- 4.15 |  |
| 37.6 +- 5.63                        | 33.5 +- 7.42           | 32.2 +- 6.32     |               |              |  |
| 2                                   | Sample 1 sap           | 800              | 36.6 +- 1.96  | 37.1 +- 9.74 |  |
| 36.4 +- 4.81                        | 28.1 +- 1.83           | 30.4 +- 4.75     |               |              |  |
| 3                                   | Sample 1 sap           | 1600             | 43.4 +- 10.53 | 35.9 +- 3.84 |  |
| 38.4 +- 4.56                        | 31.1 +- 4.47           | 36.5 +- 5.39     |               |              |  |
| 4                                   | Sample 1 sap           | 3200             | 40.1 +- 5.58  | 33.3 +- 3.01 |  |
| 37.3 +- 4.46                        | 31.3 +- 3.48           | 31.7 +- 3.18     |               |              |  |
| 5                                   | Sample 1 sep           | 6400             | 43.8 +- 8.57  | 36.3 +- 9.02 |  |
| 35.4 +- 8.18                        | 34.0 +- 6.62           | 35.1 +- 5.72     |               |              |  |
| 6                                   | Sample 1 sap           | 10000            | 37.4 +- 5.34  | 32.7 +- 3.35 |  |
| 33.2 +- 4.86                        | 29.0 +- 5.11           | 32.2 +- 3.27     |               |              |  |
| 7                                   | Sample 2 spray-dried   | 38               | 40.0 +- 4.35  | 35.8 +- 4.92 |  |
| 34.7 +- 3.20                        | 30.2 +- 1.88           | 31.4 +- 2.98     |               |              |  |
| 6                                   | Sample 2 spray-dried   | 76               | 38.6 +- 1.98  | 37.0 +- 1.95 |  |
| 48.8 +- 21.5                        | 31.6 +- 4.56           | 39.0 +- 17.27    |               |              |  |
| 9                                   | Sample 3 active moiety | 2.5              | 42.0 +- 6.70  | 37.0 +- 3.12 |  |
| 5.05                                | 34.1 +- 3.16           | 28.0 +- 2.58     | 31.6 +- 3.12  |              |  |
| 10                                  | Sample 3 activo moiety | 5.0              | 40.9 +- 4.48  | 34.2 +- 3.00 |  |
| 3.00                                | 32.7 +- 1.26           | 28.2 +- 1.65     | 33.1 +- 14.82 |              |  |
| 11                                  | Fonfluramine           | 7.5              | 47.0 +- 5.3   | 35.5 +- 7.49 |  |
| 34.7 +- 3.73                        | 30.9 +- 2.12           | 31.6 +- 2.80     |               |              |  |
| 12                                  | Vehicle (DMSO)         | --               | 43.3 +- 6.67  | 34.5 +- 4.97 |  |
| 35.2 +- 4.34                        | 28.3 +- 4.64           | 31.4 +- 6.44     |               |              |  |

Effects of oral administration on water consumption in the rat (daily post-dose data)

|                                     |                 | Group mean water |              |              |              |
|-------------------------------------|-----------------|------------------|--------------|--------------|--------------|
| consumption (g +- sd) between Days: |                 |                  |              |              |              |
| Group                               | Oral treatment  | Dose (mg/kg)     | 1-2          | 2-3          | 3-4          |
| 4-5                                 | 5-6             | 6-7              | 7-8          |              |              |
| 1                                   | Vehicle (water) | --               | 34.9 +- 5.45 | 36.9 +- 6.06 | 38.0 +- 7.59 |
| 37.2 +- 6.16                        | 37.7 +- 5.54    | 35.3 +- 2.86     | 36.5 +- 5.85 |              |              |

|    |                        |       |      |       |       |       |       |       |      |       |      |    |       |
|----|------------------------|-------|------|-------|-------|-------|-------|-------|------|-------|------|----|-------|
| 2  | Sample 1 sap           | 800   | 30.9 | +-    | 3.77  | 34.4  | +-    | 8.12  | 38.2 |       |      |    |       |
| +- | 13.71                  | 35.9  | +-   | 13.51 | 39.5  | +-    | 11.20 | 28.8  | +-   | 1.22  | 31.8 | +- | 5.58  |
| 3  | Sample 1 sap           | 1600  | 29.2 | +-    | 1.66  | 31.7  | +-    | 5.35  | 41.3 |       |      |    |       |
| +- | 11.21                  | 34.8  | +-   | 4.10  | 48.1  | +-    | 12.27 | 37.8  | +-   | 7.28  | 36.9 | +- | 9.28  |
| 4  | Sample 1 sap           | 3200  | 35.9 | +-    | 5.68  | 26.2* | +-    | 2.66  | 30.5 |       |      |    |       |
| +- | 2.44                   | 34.1  | +-   | 4.80  | 45.8  | +-    | 16.54 | 51.0  | +-   | 35.21 | 42.6 | +- | 13.88 |
| 5  | Sample 1 sap           | 6400  | 33.4 | +-    | 12.04 | 27.4* | +-    | 8.13  | 32.6 |       |      |    |       |
| +- | 10.67                  | 35.4  | +-   | 10.78 | 45.2  | +-    | 8.72  | 36.2  | +-   | 6.72  | 35.9 | +- | 9.58  |
| 6  | Sample 1 sap           | 10000 | 31.7 | +-    | 12.74 | 28.5* | +-    | 8.65  | 32.4 |       |      |    |       |
| +- | 8.87                   | 36.6  | +-   | 6.50  | 40.7  | +-    | 11.51 | 38.0  | +-   | 8.66  | 37.5 | +- | 6.21  |
| 7  | Sample 2 spray-dried   | 38    | 36.0 | +-    | 6.02  | 34.5  | +-    | 1.79  | 36.2 |       |      |    |       |
| +- | 7.16                   | 39.6  | +-   | 7.09  | 42.7  | +-    | 9.74  | 45.6  | +-   | 17.15 | 46.1 | +- | 9.49  |
| 8  | Sample 2 spray-dried   | 76    | 45.0 | +-    | 19.03 | 39.1  | +-    | 16.59 | 46.9 |       |      |    |       |
| +- | 18.34                  | 35.9  | +-   | 3.40  | 41.9  | +-    | 12.37 | 36.9  | +-   | 6.47  | 38.1 | +- | 6.93  |
| 9  | Sample 3 active moiety | 2.5   | 32.2 | +-    | 4.01  | 36.1  | +-    | 12.42 | 38.3 |       |      |    |       |
| +- | 11.71                  | 41.5  | +-   | 16.60 | 34.7  | +-    | 7.57  | 33.0  | +-   | 4.20  | 35.3 | +- | 8.70  |
| 10 | Sample 3 active moiety | 5.0   | 33.9 | +-    | 2.40  | 31.5  | +-    | 8.12  | 35.1 |       |      |    |       |
| +- | 3.82                   | 37.7  | +-   | 5.99  | 39.5  | +-    | 7.78  | 37.4  | +-   | 11.07 | 37.8 | +- | 6.42  |
| 11 | Fenfluramine           | 7.5   | 34.1 | +-    | 3.60  | 37.2  | +-    | 1.48  | 36.7 |       |      |    |       |
| +- | 3.92                   | 33.6  | +-   | 2.89  | 33.7  | +-    | 5.43  | 32.1  | +-   | 1.93  | 33.6 | +- | 2.50  |
| 12 | Vehicle (DMSO)         | --    | 40.7 | +-    | 9.10  | 33.8  | +-    | 9.37  | 32.8 |       |      |    |       |
| +- | 7.07                   | 35.8  | +-   | 11.49 | 33.8  | +-    | 9.62  | 32.3  | +-   | 7.44  | 32.0 | +- | 7.22  |

sd Standard deviation

Groups 2-8 were compared with vehicle Group 1: \*p < 0.05

Groups 9-11 were compared with vehicle Group 12 (no significances)

TABLE 3

Effects of oral administration on bodyweight in the rat (daily pre-dose data)

| bodyweight (g +- sd) on Day: |                        | Group mean     |                              |
|------------------------------|------------------------|----------------|------------------------------|
| Group                        | Oral treatment         | Dose (mg/kg)   |                              |
| -3                           | -2                     | -1             |                              |
| 1                            | Vehicle (water)        | --             | 130.9 +- 5.56 150.7 +- 6.70  |
| 5.37                         | 157.3 +- 5.29          | 168.1 +- 6.20  | 177.5 +- 6.70                |
| 2                            | Sample 1 sap           | 800            | 131.6 +- 4.34 150.1 +- 4.10  |
| 4.84                         | 158.5 +- 4.35          | 169.6 +- 4.99  | 177.7 +- 4.10                |
| 3                            | Sample 1 sap           | 1600           | 130.1 +- 4.3 148.6 +- 6.37   |
| 6.59                         | 156.7 +- 6.38          | 167.5 +- 6.04  | 176.6 +- 6.37                |
| 4                            | Sample 1 sap           | 3200           | 130.8 +- 6.19 147.7 +- 9.10  |
| 7.56                         | 154.4 +- 8.06          | 165.2 +- 8.43  | 175.8 +- 9.10                |
| 5                            | Sample 1 sap           | 6400           | 132.6 +- 7.01 151.3 +- 7.75  |
| 7.23                         | 158.4 +- 8.50          | 169.0 +- 8.79  | 178.1 +- 7.75                |
| 6                            | Sample 1 sap           | 10000          | 132.3 +- 6.75 151.8 +- 10.90 |
| 9.08                         | 157.3 +- 9.37          | 167.1 +- 10.41 | 175.4 +- 10.90               |
| 7                            | Sample 2 spray-dried   | 38             | 131.7 +- 8.28 149.0 +- 8.42  |
| 5.85                         | 156.2 +- 5.81          | 166.7 +- 5.54  | 175.6 +- 8.42                |
| 8                            | Sample 2 spray-dried   | 76             | 130.0 +- 6.99 146.1 +- 6.55  |
| 6.00                         | 155.9 +- 6.59          | 166.0 +- 6.87  | 175.1 +- 6.55                |
| 9                            | Sample 3 active moiety | 2.5            | 132.6 +- 7.63 158.9 +- 8.71  |
| 8.51                         | 157.3 +- 8.91          | 169.8 +- 8.96  | 179.4 +- 8.71                |
| 10                           | Sample 3 active moiety | 5.0            | 133.5 +- 6.45 150.5 +- 9.20  |
| 9.55                         | 158.8 +- 8.48          | 171.0 +- 7.72  | 179.0 +- 9.20                |
| 11                           | Fenfluramine           | 7.5            | 133.2 +- 9.21 152.7 +- 10.21 |
| 9.09                         | 160.0 +- 9.82          | 170.0 +- 9.15  | 182.8 +- 10.21               |
| 12                           | Vehicle (DMSO)         | --             | 129.1 +- 3.17 147.3 +- 8.26  |
| 4.37                         | 155.0 +- 6.29          | 166.0 +- 5.91  | 174.8 +- 8.26                |

Effects of oral administration on bodyweight in the rat (daily post-dose data)

| Group | Oral treatment | Dose (mg/kg) | Pre-dose (1) | 2 | 3 |
|-------|----------------|--------------|--------------|---|---|
| 4     | 5              | 6            | 7            | 8 |   |

|       |                        |       |       |       |         |
|-------|------------------------|-------|-------|-------|---------|
| 1     | Vehicle (water)        | --    | 185.4 | 192.6 | 202.0   |
|       |                        |       | 7.77  | 7.16  | 10.17   |
| 7.98  | 10.35                  | 10.26 | 11.62 | 11.97 |         |
| 2     | Sample 1 sap           | 800   | 186.0 | 187.8 | 198.5   |
|       |                        |       | 4.90  | 4.55  | 4.20    |
| 5.91  | 4.65                   | 4.99  | 3.70  | 3.65  |         |
| 3     | Sample 1 sap           | 1600  | 185.0 | 181.0 | 193.2   |
|       |                        |       | 6.67  | 8.28  | 6.42    |
| 6.40  | 5.61                   | 6.33  | 7.70  | 6.86  |         |
| 4     | Sample 1 sap           | 3200  | 181.8 | 184.6 | 186.2*  |
|       |                        |       | 11.18 | 8.88  | 6.67    |
| 9.99  | 9.34                   | 10.21 | 11.29 | 12.18 |         |
| 5     | Sample 1 sap           | 6400  | 166.6 | 185.6 | 183.8** |
|       |                        |       | 7.96  | 6.39  | 6.67    |
| 9.16  | 7.69                   | 6.69  | 6.98  | 7.94  |         |
| 6     | Sample 1 sap           | 10000 | 182.8 | 181.4 | 179.8** |
|       |                        |       | 12.22 | 14.06 | 15.85   |
| 13.85 | 11.28                  | 10.99 | 11.68 | 11.35 |         |
| 7     | Sample 2 spray-dried   | 38    | 183.4 | 185.8 | 195.8   |
|       |                        |       | 11.11 | 9.23  | 7.79    |
| 9.79  | 9.61                   | 9.34  | 10.62 | 11.46 |         |
| 8     | Sample 2 spray-dried   | 76    | 160.6 | 163.4 | 188.6*  |
|       |                        |       | 6.47  | 7.57  | 6.73    |
| 8.50  | 9.43                   | 9.51  | 9.49  | 9.66  |         |
| 9     | Sample 3 active moiety | 2.5   | 166.2 | 191.2 | 200.0   |
|       |                        |       | 9.42  | 11.15 | 11.25   |
| 12.28 | 12.95                  | 13.69 | 14.50 | 14.35 |         |
| 10    | Sample 3 active moiety | 5.0   | 186.4 | 192.0 | 192.4   |
|       |                        |       | 10.02 | 9.93  | 9.64    |
| 11.27 | 12.70                  | 11.66 | 12.26 | 13.65 |         |
| 11    | Fenfluramine           | 7.5   | 190.3 | 190.3 | 197.7   |
|       |                        |       | 9.71  | 10.97 | 7.37    |
| 7.23  | 10.69                  | 10.12 | 12.70 | 9.24  |         |
| 12    | Vehicle (DMSO)         | --    | 183.3 | 190.3 | 199.0   |
|       |                        |       | 8.33  | 10.26 | 10.82   |
| 14.05 | 11.64                  | 15.95 | 17.35 |       |         |

sd Standard deviation

Groups 2-8 were compared with vehicle Group 1: \*p < 0.05, \*\*p < 0.01

Groups 9-11 were compared with vehicle Group 12 (no significances)

### Histopathology Report

Histological examination was restricted to the liver. No treatment-related changes were detected for Sample 1 (liquid), Sample 2 (spray-dried sap), Sample 3 (active moiety), fenfluramine or the DMSO

control group.

The findings recorded were of a similar incidence in control and treated groups.

TABLE  
Microscopic pathology incidence summary

| Group 4  | Group 5    | Group 6     | Group 1 | Group 2   | Group 3    |      |
|--|------------|-------------|---------|-----------|------------|------|
| Sex: Males<br>mg/kg                            | 6400 kg/mg | 10000 mg/kg | 0 mg/kg | 800 mg/kg | 1600 mg/kg | 3200 |
| Males on study                                 |            |             | 5       | 4         | 5          |      |
| 5  | 5          | 5           |         |           |            |      |
| Animals completed                              |            |             | 5       | 4         | 5          |      |
| 5  | 5          | 5           |         |           |            |      |
| Liver  |            |             |         |           |            |      |
|  |            | 5           |         |           |            |      |
| Examined                                       |            |             | 5       | 4         | 5          |      |
| 5  | 5          | 0           |         |           |            |      |
| No abnormalities detected                      |            |             | 0       | 0         | 1          |      |
| 2  | 3          | 3           |         |           |            |      |
| Parenchymal inflammatory cell foci (Total)     |            |             | 0       | 1         | 0          |      |
| 0  | 0          | 3           |         |           |            |      |
| Minimal  |            |             | 0       | 1         | 0          |      |
| 0  | 0          | 1           |         |           |            |      |
| Hepatocyte hypertrophy - centrilobular (Total) |            |             | 0       | 0         | 0          | 0    |
| 0  | 0          | 1           |         |           |            |      |
| Minimal  |            |             | 0       | 0         | 0          |      |
| 0  | 0          | 0           |         |           |            |      |
| Extramedullary haemopoiesis (Total)            |            |             | 2       | 0         | 0          |      |
| 0  | 0          | 0           |         |           |            |      |
| Minimal  |            |             | 2       | 0         | 0          |      |
| 0  | 0          | 0           |         |           |            |      |
| Hepatocyte necrosis - focal (Total)            |            |             | 1       | 0         | 0          |      |
| 0  | 0          | 0           |         |           |            |      |
| Minimal  |            |             | 1       | 0         | 0          |      |
| 0  | 0          | 0           |         |           |            |      |
| Portal lymphoid infiltration (Total)           |            |             | 3       | 4         | 4          |      |
| 3  | 2          | 2           |         |           |            |      |
| Minimal  |            |             | 3       | 4         | 4          |      |
| 3  | 2          | 2           |         |           |            |      |
| Eosinophilic hepatocytes - focal (Total)       |            |             | 1       | 0         | 0          |      |
| 0  | 0          | 0           |         |           |            |      |
| Minimal  |            |             | 1       | 0         | 0          |      |
| 0  | 0          | 0           |         |           |            |      |
| Portal fibrosis (Total)                        |            |             | 0       | 0         | 1          |      |
| 0  | 0          | 0           |         |           |            |      |
| Minimal  |            |             | 0       | 0         | 1          |      |
| 0  | 0          | 0           |         |           |            |      |
| Liver (ORO stain)                              |            |             |         |           |            |      |
| Examined                                       |            |             | 5       | 4         | 5          |      |
| 5  | 5          | 5           |         |           |            |      |
| No abnormalities detected                      |            |             | 2       | 3         | 2          |      |
| 4  | 3          | 3           |         |           |            |      |
| Hepatocyte fat - centrilobular (Total)         |            |             | 3       | 1         | 2          |      |
| 1  | 2          | 2           |         |           |            |      |
| Minimal  |            |             | 3       | 1         | 2          |      |
| 1  | 2          | 2           |         |           |            |      |
| Hepatocyte fat - periportal (Total)            |            |             | 0       | 0         | 1          |      |
| 0  | 0          | 0           |         |           |            |      |

|  | Group 10  | Group 11 | Group 12 | Group 7  | Group 8  | Group 9   |
|--|-----------|----------|----------|----------|----------|-----------|
| Minimal                                    | 0         | 0        | 0        | 0        | 0        | 1         |
| Sex: Males                                 |           |          |          | 38 mg/kg | 76 mg/kg | 2.5 mg/kg |
| mg/kg                                      | 7.5 mg/kg | 0 mg/kg  |          |          |          | 5         |
| Males on study                             | 5         | 3        | 3        | 5        | 5        | 5         |
| Animals completed                          | 5         | 3        | 3        | 5        | 5        | 5         |
| Liver                                      |           |          |          |          |          |           |
| Examined                                   | 5         | 3        | 3        | 5        | 5        | 5         |
| No abnormalities detected                  | 1         | 0        | 2        | 2        | 2        | 0         |
| Parenchymal inflammatory cell foci (Total) | 0         | 0        | 1        | 0        | 0        | 0         |
| Minimal                                    | 0         | 0        | 1        | 0        | 0        | 0         |
| Hepatocyte necrosis - focal (Total)        | 0         | 0        | 0        | 0        | 0        | 1         |
| Minimal                                    | 0         | 0        | 0        | 0        | 0        | 1         |
| Portal lymphoid infiltration (Total)       | 4         | 3        | 1        | 3        | 3        | 5         |
| Minimal                                    | 4         | 3        | 1        | 3        | 3        | 5         |
| Portal leucocytes (Total)                  | 0         | 0        | 0        | 0        | 0        | 1         |
| Minimal                                    | 0         | 0        | 0        | 0        | 0        | 1         |
| Liver (ORO stain)                          |           |          |          |          |          |           |
| Examined                                   | 5         | 3        | 3        | 5        | 5        | 5         |
| No abnormalities detected                  | 3         | 2        | 2        | 5        | 3        | 3         |
| Hepatocyte fat - centrilobular (Total)     | 2         | 1        | 1        | 0        | 2        | 2         |
| Minimal                                    | 2         | 1        | 0        | 0        | 2        | 2         |

#### EXAMPLE 45

A further bioassay, which employed the same test samples as described in Example 44, is described below. Animals in this study received a restricted diet i.e. animals only received food between 12:00 and 3:00 pm daily. This is different from all other biological assays conducted thus far, whereby food was available to the rats at lib. Animals were acclimatized over a seven day period (days -7 to -1), dosing took place from day 0 to day 6 at 9:00 am by oral gavage. The recovery period was from days 7 to day 13. Dosage groups are described in Table 1 below. It should be noted that the actual control group is labelled Group 09. Group 5 is a controlled group which received a diet equivalent to that of Group 4. The purpose of this group was to evaluate the effect a restricted diet has on the lives of the animals.

#### Results

The results generated during the study showed that the acclimatization period was too short. Rats feed

mainly during the night and the sudden change to a restricted access to feed for 3 hours during day-time, resulted in low daily intakes. The daily intake of feed was still increasing in most groups at the end of the acclimatization period when dosing with the test items started. As a result of this, the effect of the test materials did not significantly affect the food intake of the rats during the period of dosing.

The mean body masses for the different groups for day -7 to -1 and days 0 to 6 are shown in the Table D1 and Table D2. The effect of the different dosages of the sap and spray-dried sap is shown in the accompanying graphs as a change in body mass day 0 to 7 (FIG. 5), and % change in body mass day -7 to 7 (FIG. 6). The loss in body mass is clearly dose-related especially with the higher dosages.

The histopathological examination of the livers did not show any significant pathology in the groups receiving the cast items.

Food

Food consumption was measured daily, during acclimatization and during the study. Food was available for a 3 hour feeding period daily, starting at 12:00 and ending at 15:00. The animals were fasted for the remainder of the time. Animals in Group 5 received a measured quantity food on Day 1, equivalent to the average food consumption of Group 4 on Day 0. This controlled feeding pattern for Group 5, as determined from the average food consumption of Group 4 from the previous day, was followed for Days 1-7.

Water

Water was provided in standard containers. Water (Magalies Water Board Tap Water, suitable for human consumption) was available ad libitum. Water consumption was measured once daily, at the same time each day, after food consumption determination.

Acclimatization

The animals were acclimatized for seven days before the start of the study, during which time food and water consumption were determined as described above. The body masses were determined on a daily basis during this time.

Study Design and Procedures

TABLE 1  
STUDY DESIGN

| GROUP | TEST    | NUMBERS | DOSE       | TEST ITEM                       |
|-------|---------|---------|------------|---------------------------------|
| 01    | 6.male. | 001-006 | 100 mg/kg  | Frozen sap                      |
| 02    | 6.male. | 007-012 | 400 mg/kg  | Frozen sap                      |
| 03    | 6.male. | 013-018 | 1600 mg/kg | Frozen sap                      |
| 04    | 6.male. | 019-024 | 3200 mg/kg | Frozen sap                      |
| 05    | 6.male. | 025-030 | CONTROL    | Elga Option 4<br>Purified Water |
| 06    | 6.male. | 031-036 | 2.2 mg/kg  | Spray-dried sap                 |
| 07    | 6.male. | 037-042 | 8.8 mg/kg  | Spray-dried sap                 |
| 08    | 6.male. | 043-048 | 35 mg/kg   | Spray-dried sap                 |
| 09    | 6.male. | 049-054 | CONTROL    | Elga Option 4<br>Purified Water |

Route of Administration

The test items were administered on a daily basis for seven days, using an intra-gastric needle. Animals were fasted for 8 hours prior to the item administration (starting at 09:00).

Duration of Treatment

Animals were treated for seven consecutive days (from Day 0-Day 6). Three animals of each group were sacrificed 24 hours after the last dosing (Day 7). The remaining three animals were sacrificed 7 days after the last treatment (Day 13). This procedure was followed for all the groups except for Group 5 where three animals were sacrificed 24 hours after the last controlled feeding (Day 8), the remaining three animals were sacrificed 7 days after the last treatment (Day 13).

Body Masses

Body masses were determined daily, at approximately the same time each day for the duration of the study, including during the acclimatization period.

Euthanasia

Three animals of each group were sacrificed 24 hours after the last dosing (Day 7).

The remaining three animals were sacrificed 7 days after the last treatment. This procedure was followed for all the groups except for Group 5 where three animals were sacrificed 24 hours after the last controlled feeding (Day 8), the remaining three animals were sacrificed 7 Days after the last treatment (Day 13). The animals were euthanased at the end of the study period with CO.sub.2 gas.

Ophthalmoscopic Examinations

Ophthalmoscopic examinations, using an ophthalmoscope, were done prior to the first administration of the test item and at termination, in all animals in all groups.

Macroscopic Pathology

A full post mortem examination was performed on every animal which was euthanased at the end of the study period.

Histopathology

Histopathological examination was performed on the liver of each of the animals.

TABLE D  
MEAN BODY MASSES/GROUP 1 WEEK

| Standard deviation |                |             |             | Dose    | Mean body masses (g) & |             |             |        |
|--------------------|----------------|-------------|-------------|---------|------------------------|-------------|-------------|--------|
| Group              | Oral treatment |             |             | (mg/kg) | Day -7                 | Day -6      | Day -5      | Day -4 |
|                    | Day -3         | Day -2      | Day -1      |         |                        |             |             |        |
| 01                 | Sample 1 (Sap) |             |             | 100     | 203.38 .+-.            | 197.13 .+-. | 192.75 .+-. |        |
|                    | 188.62 .+-.    | 164.95 .+-. | 182.48 .+-. |         | 182.25 .+-.            |             |             |        |
|                    | 84.80          | 83.47       | 82.57       |         | 95.39                  | 90.63       | 89.49       | 86.75  |
| 02                 | Sample 1 (Sap) |             |             | 400     | 192.53 .+-.            | 183.92 .+-. | 178.25 .+-. |        |
|                    | 173.17 .+-.    | 170.82 .+-. | 168.25 .+-. |         | 169.37 .+-.            |             |             |        |

|        |                            |        |         |                        |        |        |       |
|--------|----------------------------|--------|---------|------------------------|--------|--------|-------|
|        |                            |        |         | 65.60                  | 61.20  | 59.37  | 58.10 |
| 57.42  | 58.40                      | 59.25  |         |                        |        |        |       |
| 03     | Sample 1 (Sap)             |        | 1600    | 149.25                 | 142.87 | 136.85 |       |
| 132.37 | 131.50                     | 129.67 |         | 131.12                 |        |        |       |
|        |                            |        |         | 54.80                  | 51.89  | 52.17  | 49.64 |
| 49.50  | 48.89                      | 48.22  |         |                        |        |        |       |
| 04     | Sample 1 (Sap)             |        | 3200    | 224.15                 | 214.45 | 207.10 |       |
| 201.82 | 198.25                     | 194.83 |         | 196.77                 |        |        |       |
|        |                            |        |         | 80.70                  | 77.25  | 76.38  | 75.42 |
| 74.82  | 75.34                      | 74.56  |         |                        |        |        |       |
| 05     | Elga Option 4              |        | --      | 214.55                 | 204.65 | 198.57 |       |
| 193.48 | 192.40                     | 190.67 |         | 190.15                 |        |        |       |
|        | purified water (control)   |        |         | 74.90                  | 72.41  | 71.79  |       |
| 68.49  | 67.48                      | 67.39  | 65.24   |                        |        |        |       |
| 06     | Sample 2 (Spray-dried sap) |        | 2.2     | 206.65                 | 199.37 | 193.18 |       |
|        |                            |        |         | 185.97                 |        |        |       |
|        |                            |        |         | 65.74                  | 62.49  | 61.18  | 60.89 |
| 59.98  | 58.86                      | 56.78  |         |                        |        |        |       |
| 07     | Sample 2 (Spray-dried sap) |        | 8.8     | 256.95                 | 246.02 | 237.47 |       |
|        |                            |        |         | 228.45                 |        |        |       |
|        |                            |        |         | 77.55                  | 73.67  | 73.53  | 71.73 |
| 71.76  | 69.88                      | 68.81  |         |                        |        |        |       |
| 08     | Sample 2 (Spray-dried sap) |        | 35      | 194.37                 | 185.83 | 777.53 |       |
|        |                            |        |         | 168.00                 |        |        |       |
|        |                            |        |         | 43.74                  | 42.70  | 41.10  | 40.13 |
| 39.49  | 37.61                      | 38.83  |         |                        |        |        |       |
| 09     | Elga Option 4              |        | --      | 171.52                 | 162.67 | 154.95 |       |
| 151.38 | 149.63                     | 148.30 |         | 149.07                 |        |        |       |
|        | purified water (control)   |        |         | 69.81                  | 62.68  | 61.83  |       |
| 59.46  | 57.66                      | 57.12  | 56.01   |                        |        |        |       |
|        |                            |        | Dose    |                        |        |        |       |
|        |                            |        |         | Mean body masses (g) & |        |        |       |
|        |                            |        |         | Standard deviation     |        |        |       |
| Group  | Oral treatment             |        | (mg/kg) | Day 0                  | Day 1  | Day 2  | Day 3 |
|        | Day 4                      | Day 5  | Day 6   |                        |        |        |       |
| 01     | Sample 1 (Sap)             |        | 100     | 183.87                 | 175.83 | 175.72 |       |
| 175.48 | 175.53                     | 177.95 |         | 178.43                 |        |        |       |
|        |                            |        |         | 83.33                  | 81.82  | 79.05  | 77.54 |
| 76.20  | 73.99                      | 72.68  |         |                        |        |        |       |
| 02     | Sample 1 (Sap)             |        | 400     | 173.45                 | 164.58 | 164.75 |       |
| 166.22 | 166.55                     | 169.93 |         | 171.77                 |        |        |       |
|        |                            |        |         | 60.73                  | 58.52  | 58.37  | 57.69 |
| 57.79  | 57.47                      | 57.29  |         |                        |        |        |       |
| 03     | Sample (Sap)               |        | 1600    | 134.38                 | 129.20 | 127.53 |       |
| 127.20 | 126.70                     | 128.00 |         | 128.07                 |        |        |       |
|        |                            |        |         | 46.01                  | 44.74  | 43.20  | 41.36 |
| 39.19  | 39.22                      | 38.66  |         |                        |        |        |       |
| 04     | Sample (Sap)               |        | 3200    | 199.60                 | 196.38 | 192.20 |       |
| 189.05 | 186.57                     | 186.05 |         | 185.68                 |        |        |       |
|        |                            |        |         | 75.16                  | 73.96  | 71.20  | 69.11 |
| 66.29  | 67.45                      | 65.73  |         |                        |        |        |       |
| 05     | Elga Option 4              |        | --      | 194.27                 | 187.93 | 181.97 |       |
| 177.53 | 174.73                     | 172.85 |         | 171.45                 |        |        |       |
|        | purified water (control)   |        |         | 67.46                  | 65.48  | 65.01  |       |
| 64.73  | 61.08                      | 58.63  | 56.79   |                        |        |        |       |
| 06     | Sample 2 (Spray-dried sap) |        | 2.2     | 189.07                 | 181.52 | 181.48 |       |
|        |                            |        |         | 189.68                 |        |        |       |
|        |                            |        |         | 60.15                  | 58.99  | 57.79  | 55.64 |
| 55.29  | 54.66                      | 53.70  |         |                        |        |        |       |
| 07     | Sample 2 (Spray-dried sap) |        | 8.8     | 230.28                 | 221.55 | 220.17 |       |
|        |                            |        |         | 224.82                 |        |        |       |
|        |                            |        |         | 224.90                 |        |        |       |



|        |   |     |     |       |
|--------|---|-----|-----|-------|
| Day 13 | 04  | NPL | MLC |       |
|        | 05  |     |     | FHS1+ |
|        | 06  | NPL |     |       |
|        | GROUP 2: 400 mg/kg Sample 1   |     |     |       |
|        | 07  |     |     | FHS1+ |
|        | 08  | NPL | C1+ |       |
|        | 09  | NPL |     |       |
| Day 13 | 10  |     |     | DHS1+ |
|        | 11  | NPL |     |       |
|        | 12  |     |     | DHS1  |
|        | GROUP 3: 1600 mg/kg Sample 1  |     |     |       |
| Day 7  | 13  | NPL |     |       |
|        | 14  | NPL |     |       |
|        | 15  | NPL |     |       |
| Day 13 | 16  | NPL |     |       |
|        | 17  |     |     | DHS1+ |
|        | 18  | NPL |     |       |
|        | GROUP 4: 3200 mg/kg Sample 1  |     |     |       |
|        | 19  | NPL |     |       |
|        | 20  | NPL |     |       |
|        | 21  | NPL |     |       |
| Day 13 | 22  |     |     | DHS1+ |
|        | 23  |     |     | FHS1+ |
|        | 24  | NPL |     |       |
|        | GROUP 5: CONTROL: ELGA OPTION 4 PURIFIED WATER:<br>RESTRICTED FOOD INTAKE |     |     |       |
|        | GROUP 5: CONTROL: Elga option purified water                              |     |     |       |
| Day 7  | 25  | NPL | MLC |       |
|        | 26  | NPL |     |       |
|        | 27  | NPL |     |       |
| Day 13 | 28  |     |     | DHS1+ |
|        | 29  |     |     | DHS1+ |
|        | 30  | NPL |     |       |

Legend:

- C = Congestion
- DHS = Diffuse hydropic cell swelling
- FHS = Focal hydropic cell swelling
- NPL = No parenchymal fesiions
- MLC = Minimal lymphocytic cuffing
- 1+ = mild
- 2+ = moderate
- 3+ = severe

TABLE 2  
HISTOLOGICAL EVALUATION  
OF LIVER SECTIONS FROM MALE RATS  
Sample 2

|        | Animal no                   | Hepatic lesions |     |       |
|--------|-----------------------------|-----------------|-----|-------|
|        | GROUP 6: 2.2 mg/kg Sample 2 |                 |     |       |
| Day 7  | 31                          | NPL             |     |       |
|        | 32                          | NPL             | MLC |       |
|        | 33                          |                 |     | FHS1+ |
| Day 13 | 34                          | NPL             |     |       |
|        | 35                          |                 |     | DHS1+ |
|        | 36                          | NPL             |     |       |
|        | GROUP 7: 8.8 mg/kg Sample 2 |                 |     |       |
|        | 37                          | NPL             |     |       |
|        | 36                          | NPL             |     |       |
|        | 39                          | NPL             | C1+ |       |
| Day 13 | 40                          |                 |     | DHS1+ |
|        | 41                          | NPL             |     |       |



advantageously administered to said human in a dosage amount of from about 0.01 mg/kg/day to about 10 mg/kg/day. A preferred dosage range is 0.05 mg/kg/day to 0.5 mg/kg/day. When using the spray dried powder form of the extract of this invention, a preferred dosage range is 0.1 mg/kg/day to 20 mg/kg/day; especially preferred is 0.5 mg/kg/day to 5 mg/kg/day.

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