

is removed; but what part of the population is responsible for the damage has not yet been ascertained. There is also evidence that there is some segregation of the population into sex- and age-groups, but much more information is required on this point. The recovery of seals marked as pups² has proved that some of them at least travel very long distances in the first few months of their lives, for example from the Farne Islands to Norway, Faroe, and the coasts of Germany, Holland, and Belgium. The movements of older animals have yet to be traced; regular tagging has been carried on for less than ten years, and it is probable that the methods used at first were not efficient, so that the seals lost their tags after a comparatively short time.

It is thus evident that no satisfactory policy about grey seals in relation to fisheries can be settled until the requisite knowledge about their natural history has been obtained. It is for this reason that the Nature Conservancy is at present sponsoring a three-year programme of research on the grey seal, financed partly by the Development Commissioners.

In a recent paper* which should form a useful background to this investigation, Dr. B. B. Rae has attempted to gather together all that has been written about the grey and common seals in relation to fisheries since the passing of the Grey Seal Protection Act in 1914. Unfortunately, Dr. Rae mixes the vague statements of untrained observers with the relatively few proved facts, and tends to reach conclusions some of which start from false premises; he sometimes appears to abandon objectivity by straining to build up a case against the grey seal with statements that are not adequately supported by evidence. There is a *prima facie* case against the grey seal, but the solution of the problems it presents can only be found by patient research and the laying aside of all emotion and bias on both sides. The work is in hand, and if faithfully and diligently prosecuted, there is no doubt that the desired result will be attained.

* Department of Agriculture and Fisheries for Scotland. Marine Research 1960, No. 2: Seals and Scottish Fisheries. By Dr. Bennett B. Rae. Pp. 39 - 2 plates. (Edinburgh and London: H.M. Stationery Office, 1960.) 15s. net.

MIRCESTROL: AN OESTROGEN FROM THE PLANT *PUERARIA MIFRIFICA*

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IN 1936, Dr. S. J. Folley, working at the National Institute for Research in Dairying at Shinfield, in England, found that the injection of oestrone or oestradiol benzoate into lactating cows caused prolonged increases in both the fat and the non-fatty solids content of the milk¹. This 'enrichment effect', as it was then called, was later confirmed and further investigated by Folley and his colleagues².

During the early spring of 1948, Dr. H. W. Bennetts, of Perth, Western Australia, discussed with Drs. Bartlett and Folley his findings that reproductive disorders in sheep were due to the ingestion of oestrogenic compounds apparently present in subterranean clover, which at that time was the principal diet of sheep in Western Australia. In view of this finding and the work reported above by Folley and his colleagues, it was decided to initiate work at the National Institute for Research in Dairying on oestrogens in pasture herbage. Dr. Bennetts kindly provided helpful information on the techniques which he had found useful, and advice and help was also sought and obtained from Sir Charles Dodds and his colleagues at the Courtauld Institute of Biochemistry, Middlesex Hospital, London, where also at that time D. H. Curnow, a research worker from Western Australia, was studying herbage oestrogens.

In May 1948 oestrogenic activity was demonstrated in the pasture herbage from a field adjoining the National Institute for Research in Dairying by Bartlett and his colleagues³. This finding posed a host of questions of practical significance for the solution of which it was clear that the resources

available at that time at the Institute and the Courtauld Institute needed to be strengthened on the chemical side. Accordingly, in 1949 Dr. G. S. Pope joined the staff at Shinfield for work on oestrogens in pasture plants; the progress of this work has already been reported⁴.

In the early stages of the work, when attempts were being made to isolate the oestrogens of red clover (aerial portions of the plant), attention was directed to the chemically new and highly potent oestrogen isolated in 1940 by Schoeller, Doorn and Hohlweg⁵ from the tuberous roots of a leguminous plant in Thailand, investigated from a chemical point of view by Butenandt⁶ (1940). It was evident that this plant source was at least a hundred times as rich in oestrogenic activity as red clover and it became of considerable interest for two reasons. The first was that since both it and red clover are of the family Leguminosae, it seemed likely that the slight activity of red clover was due to a low content of the same new oestrogen as found in the plant in Thailand, and, therefore, that study of the latter might greatly help the work with red clover. (In the event this forecast proved to be wrong, as the activity of red clover was found to be due to the very slightly potent isoflavonoid oestrogens.) The second reason was a natural interest in the new, highly potent oestrogen for its intrinsic value.

At this stage, Mr. D. C. Rivett-Carnac, of the British Embassy, Bangkok, with whom the workers at Shinfield were in touch, directed attention to certain relevant information in some of the Thai scientific journals.

ห้องสมุดกรมวิทยาศาสตร์

Kerr¹ in 1932 was the first to direct public attention to the fact that the tuberous roots of the Thai plant concerned, a woody climber found in the forests of northern Thailand and known locally as *kwao keur*, were considered to be of value as the active constituent of a rejuvenating drug. The information came to him privately from Mr. H. B. Garrett and Dr. E. C. Cort, and the conclusion reached was that the plant was *Butea superba*.² Following Kerr's paper, various workers began to collect the roots, and in 1939 Vaina³ showed the presence of oestrogen in *kwao keur*, this being confirmed by Schoeller *et al.*⁴ It became clear, however, after detailed botanical investigation in Thailand, that the name *kwao keur* was, in fact, applied to several species of plant having tuberous roots, and that those yielding the new oestrogen belonged to an unnamed species of *Pueraria*, superficially very similar to *Butea superba*. The name *Pueraria mirifica* was given to it by Lakshnakara Kashemsanta, Kasin Suvatabandhu and Airy Shaw.⁵

In 1952 a gift of 6.1 kgm. of dried *Pueraria mirifica* root was received at Shinfield from Mr. Kasin Suvatabandhu, and in 1953 and 1954 batches of 9 kgm. and 4 kgm. were again obtained through the generosity of Mr. Kasin Suvatabandhu and with the assistance of British Council staff in London and Bangkok. Bioassay, using the mouse uterine growth test, of a crude methanol extract of the powdered root confirmed the high oestrogenic activity of the material. The crude methanol extract (6 per cent by weight of dried root) was then submitted to conventional solvent partition procedures giving a product (0.5 per cent by weight of dried root) which contained more than 50 per cent of the oestrogen. Further purification was effected by partition chromatography on kieselguhr columns, and certain fractions from these crystallized in part and yielded a crystalline product (58 mgm. from 6.1 kgm. dried root) when washed with ethyl acetate. Recrystallization from methanol yielded as thin rectangular plates a colourless crystalline product, melting with decomposition at 268° C. (Kofler apparatus). This product appeared to be homogeneous when chromatographed on paper in three different solvent mixtures, in each case showing as a pink spot on reacting with 5 per cent aqueous potassium carbonate and diazotized *p*-aminophenyl 2-diethylaminoethyl sulphone. It appeared, therefore, that the product, of melting point 268° C., was a pure compound, and on testing for oestrogenic activity it was found to be equal to oestradiol-17 β in potency in the mouse uterine growth test, and to have one-quarter of the potency of oestradiol-17 β in the rat vaginal cornification test, in each case oestrogens being administered by subcutaneous injection. The name 'mircestrol' was given to the oestrogen; it is doubtless identical to the compound isolated by Schoeller *et al.*⁴

For further biological and chemical work, and especially for an attack on the structure of mircestrol, much larger quantities of the tuberous roots were required. Dr. S. Bartlett was responsible for the arrangements eventually made to collect additional supplies of the root, and he personally supervised the collections on site. The only place where the plant was known to grow was in the forest near Chiangmai in northern Thailand, and it was clear that supplies could only be obtained by means of a collaborative effort with the appropriate local authorities. Those who gave generous help and advice included Prince Lakshnakara Kashemsanta, Mr. Kasin Suvatabandhu, the director and staff of the Maejo Agricultural

Experimental Station at Chiangmai, and Mr. R. J. Hilton, head of the British Council Department in Bangkok.

At this time the interest of the National Research Development Corporation was sought and obtained in this work. The chemical novelty was certain at this stage; the point was whether the structure, when known, might be readily accessible by synthesis from known and abundant substances. The Corporation accordingly undertook to obtain whatever patent protection was possible in the light of prior knowledge and publication and agreed to finance an additional chemist, Dr. D. G. Bounds, at Shinfield, and certain other costs likely to be incurred in the collection of the root, the extraction of mircestrol and the determination of its structure.

Two plant-collecting expeditions to Thailand were then undertaken by Dr. Bartlett. The first in 1954, yielding 120 kgm. of dried root, was financed mainly by a grant from Quaker Oats, Ltd., but in part by the National Research Development Corporation, and the second in 1957, which was wholly financed by the latter, yielded 200 kgm.

Mr. Suvatabandhu, a well-informed Thai botanist, knows of only two places in Thailand where quantities of the plant *Pueraria mirifica* grow: one place is at the foot of the mountain Doi Suthep near Chiangmai, and the other is nearly 100 miles farther north at Chiang Dao.

The plant clearly has a local reputation as a rejuvenator. At Chiang Dao, Dr. Bartlett and his Thai colleagues were travelling by car in search of the plant and asked one of the men who were repairing the road if he knew the plant, and he replied, "Yes, we eat it!" The tubers of the plant are almost flavoured and somewhat turnip-like in consistency, and as the Thais are not short of food it may be assumed that *Pueraria mirifica* is eaten for some purpose other than as ordinary food. Further evidence of local interest in the plant may be gleaned from the fact that Mr. Puk Pakkasem, a member of the office staff at the British Consulate at Chiangmai, was able, when requested, to obtain in a few hours thirty local inhabitants who were able to locate the plant in the forest and dig it.

An interesting episode occurred in November 1954, when Dr. Bartlett visited the Siam City Bank in Chiangmai accompanied by Mr. Suvatabandhu. During casual conversation the bank manager, Mr. Kraisi Nimnanahaemino, showed great interest in the fact that they were collecting *kwao* and said his grandfather used to make medicine from the plant and had written a pamphlet about it. Mr. Nimnanahaemino provided a copy of the pamphlet and a pot of 'medicine', which appeared to be a 50:50 mixture of dried *kwao* and honey; it was made in 1953. The pamphlet, in the Thai language, was kindly translated by Mr. Stuart Simmonds, of the School of Oriental and African Studies, University of London, and gave the following information: On the cover was a diagrammatic but accurate sketch of the plant and the description: "Treatise on the Drug made from the Tubers of the Kwao Vine by Luang Anusar Sunthon, Special Commissioner, Chiangmai. Printed at the Upati Pong Printing House, 3000 copies, May 1931". The main source of information for the pamphlet was a treatise written on palm leaves by 'ancients' and stored in a temple at Pagan, the old capital of Burma. The temple was wrecked by lightning, and the treatise then came to light.

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The drug is said to be well known in Burma, where it is called 'paukse', and is stated to rejuvenate old men and women, but should not be taken by young people. One man who took the drug was reputed to have lived to 250 years of age. This local folklore, which appears to ascribe remarkable properties to the *kwao* (or *Pueraria mirifica*) in Burma and Thailand, may well be responsible for the comparative scarcity of the plant. It is not known whether it grows in Burma, but the area of Pagan mentioned in the pamphlet is now said to be an arid desert due to the forest having been cut down in order to build temples.

The pharmaceutical properties of *Pueraria* are considered, in Thailand, to include toxicity, and a careful study of the constituents of the plant has been made in the Chemistry Department of the University of Bangkok. At the Ninth Pacific Science Congress at Bangkok in November 1957, an interesting paper was presented on the "Constituents of the Tuberous Roots of *Pueraria mirifica*" by the Thai workers, T. Nilanidhi, B. Kamthong, K. Isarasona and D. Shienthong (unpublished work), in addition to a joint one by the Thai and United Kingdom workers, Lakshnakara Kashemsanta, Kasin Svatabandhu, Bartlett and Pope, on "The Oestrogenic Substance (Miracestrol) from the Tuberous Roots of *Pueraria mirifica*"¹⁰.

The collaboration of the Director of the Royal Botanic Gardens at Kew was sought in an attempt to grow plants of *Pueraria mirifica* there. The first two attempts were unsuccessful, but the third plant, kindly sent from Thailand by Col. E. H. Jacobs-Larkcom, grew and is still growing vigorously. In December 1953 Miss Joy Jacobs-Larkcom collected a number of additional plants in Chiangmai, and these are now also planted in Kew Gardens.

The roots obtained by the expeditions to Thailand were utilized for the following purposes in the studies on miracestrol.

(a) *Improvement of the method of isolation.* The method of isolation has been simplified, and recovery of around 15 mgm. miracestrol per kgm. dried root is now a routine procedure. Paper chromatography, using periodate as reagent, which provides a very sensitive and almost specific colour test for miracestrol, gives efficient analytical control.

(b) *Determination of physical properties.* Miracestrol has been found to crystallize in two forms, a stable hydrated form (stout needles), which after desiccation quickly regains its water of crystallization when exposed to moist air, and a non-hygroscopic, anhydrous form (thin rectangular plates). The latter form has a sharp melting point of 268° C., with some decomposition on melting (Kofler apparatus, rheostat setting 350° C.). Like many steroid hormones, miracestrol has a high dextrorotatory power, $[\alpha]_D^{25} + 301^\circ$ (1.08 gm./100 ml. in ethanol). Its ultra-violet and infra-red absorption curves have also been useful criteria of the purity of the various specimens isolated.

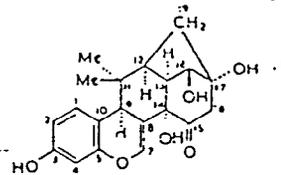
(c) *Determination of oestrogenic potency.* In addition to the properties already noted, miracestrol has been found to have high oestrogenic potency when given orally: approximately three times that of stilbestrol in the immature mouse uterine growth test¹¹ and two-thirds that of stilbestrol in the rat vaginal cornification test. It has also 70 per cent of the activity of oestradiol-17 β when administered by subcutaneous injection, in promoting mammary duct growth in the rat, and is 2.2 times as active as oestrone by a similar test in the mouse¹².

(d) *Preliminary chemical studies.* Elementary analysis and molecular weight determination in Dr. Dorothy Hodgkin's laboratory, University of Oxford, using the X-ray method, gave C₂₀H₃₂O₆ as the molecular formula for miracestrol, or less probably C₂₀H₃₀O₆. Analysis of the monomethyl ether and monoacetate, in each case the phenolic hydroxyl (see below) having reacted, supported this finding. Other derivatives of miracestrol were then prepared, details of which have been reported elsewhere¹³, and from the ultra-violet and infra-red absorption curves of these and their chemical properties it was concluded that miracestrol is a monohydric phenol possessing one carbonyl and at least two alcoholic hydroxyl groups.

(e) *Preparation of a derivative of miracestrol for X-ray analysis.* The complete elucidation of the structure of miracestrol solely by chemical methods appeared likely to be very difficult with the small amount of material available. It was therefore decided to employ X-ray crystallographic analysis to solve the problem, using three-dimensional methods on a heavy-atom derivative. This work was carried out by Dr. Dorothy Hodgkin and her colleagues at Oxford.

Dr. D. G. Bounds succeeded in making a monobromo-substitution product of miracestrol. At first, bromination of miracestrol with one molecular proportion of bromine gave complex mixtures, but reaction conditions were eventually found which gave a mixture containing only two main products. These were isolated by partition chromatography, and from the elementary analysis and ultra-violet and infra-red absorption curves of one of these compounds, it was concluded that in this compound a single bromine atom had entered the miracestrol molecule replacing a hydrogen atom and that no other chemical changes had taken place. The fact that the monobromomiracestrol isolated coupled much less readily than did miracestrol with diazotized *p*-aminophenyl 2-diethylaminoethyl sulphone was taken as good evidence that the bromine atom was present as a substituent in the benzene ring of miracestrol.

(f) *X-ray studies.* X-ray crystallographic measurements on monobromomiracestrol were made by Dr. Noel E. Taylor, and the crystal structure was solved in collaboration with Dr. Hodgkin and Dr. J. S. Rollet through a series of three-dimensional electron density calculations carried out on *Deuce* at the National Physical Laboratory. The positions of the atoms found by these calculations are illustrated in Figs. 1 (a) and (b). They lead to the structural formula I, for miracestrol itself:



(1)

Full details of this work have been reported elsewhere¹⁴.

Biogenetically, it seems possible to dissect the nucleus into an isoprene unit and a partly reduced isoflavene nucleus. This dissection relates it, on one hand, to the many plant products in which isoprene units are found linked to condensed ring systems (cf.

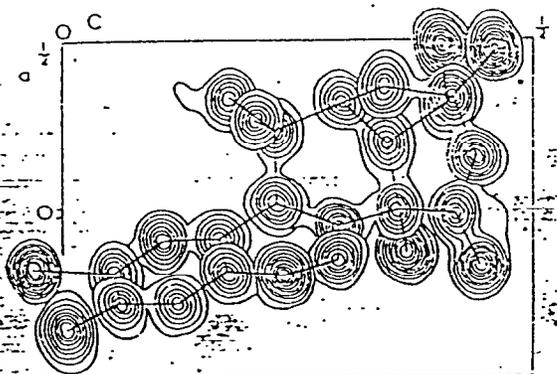


Fig. 1 (a). Electron density-levels over the peaks in p_3 in bromomiracstrol. The structure is seen projected along [010]. Contours at intervals of approximately 1 e.l./A.³ except over the bromine atom, where the intervals are 4 e.l./A.³

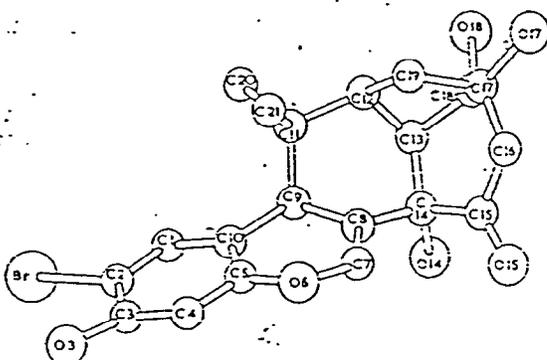


Fig. 1 (b). A view of the molecule of bromomiracstrol, drawn from the atomic positions in p_3

Robinson¹³), and, on the other, to those isoflavones and isoflavones which have been shown to have oestrogenic properties. Miracstrol is not a steroid, and the biological activity is probably a consequence of accidental features of molecular geometry. The actual distance from the 3-OH in miracstrol to the 18-OH is 10.3 Å., which is very near the distance to be expected from models of the 3-OH to the 17-OH of oestradiol. When, however, one compares the disposition of the 17-OH of miracstrol and the 16-OH of oestriol relative in each case to the 3-OH, the similarity is not so close; but perhaps it is worth noticing in this connexion that miracstrol is the more potent oestrogen.

(g) *Limited clinical trial.* The trial was carried out by Dr. P. M. F. Bishop and his colleagues at the Chelsea Hospital for Women, London.

Miracstrol has been administered to ten women suffering from amenorrhœa (primary, two cases; secondary, seven cases) or artificial menopause (one case). 5 mgm. daily was given on six occasions and 1 mgm. daily on another six occasions. Marked oestrogen response was noted with both these doses on the vaginal smear, the maximum rise in the cornification index usually occurring during the second or third week after treatment commenced. This peak usually occurred, therefore, after the treatment had been discontinued. Oestrogen withdrawal bleeding failed to take place on six occasions (three with doses of 1 mgm. daily from 5 to 10 days; three with doses of 5 mgm. daily from 2 to 14 days), though withdrawal bleedings had usually occurred in these patients when they had been previously

treated with oestrogens. Withdrawal bleeding did take place on five occasions, however, 7-13 days after the cessation of treatment. The withdrawal interval is much longer, therefore, than with most other oral oestrogens. No toxic effects were complained of on three occasions, the doses being 1 mgm. daily for 7 days, 5 mgm. daily for 8 days and 5 mgm. daily for 14 days, respectively. Toxic effects were rather marked on the remaining nine occasions and consisted of malaise, headache, nausea and in some instances vomiting. They did not seem to be appreciably less severe on the 1-mgm. doses than on the 5-mgm. doses. Work with 0.1-mgm. doses is being undertaken. On four occasions there was enlargement and tenderness of the breasts. These changes were more severe and more frequent on the 5-mgm. doses. On two occasions they were accompanied by marked pigmentation of the nipples, which persisted for some time. In the patient who had received an artificial menopause the hot flushes diminished in frequency and severity for the first week of the 14-day course, but began to return by the fifth day after treatment had been discontinued.

In the light of the limited experience described, it would seem that miracstrol produced marked oestrogenic response in the vaginal smear; is rather slow to act and its effect takes some time to wear off. It does not always induce a withdrawal bleeding, and in the doses so far employed is apt to give rise to rather disagreeable toxic effects.

In view of the undesirable side-effects of miracstrol, it appears unlikely that this substance itself will be suitable for use in medicine in spite of its high level of oestrogenic activity by the oral route. It is possible that modifications of its structure might lead to useful oestrogens, but it is evident that a total synthesis of the whole molecule would be difficult, and not likely to be commercially feasible; also although the miracstrol structure is new, it is, as noted above, related in part to already known oestrogens, and therefore does not display as great a novelty in regard to its structure-action relationship as was at first thought possible; nevertheless, miracstrol presents some features of interest, and further investigations of its biological properties are proceeding.

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