BIOLOGICAL COUNCIL

THE CO-ORDINATING COMMITTEE FOR SYMPOSIA ON DRUG ACTION

A Symposium on

AGENTS AFFECTING FERTILITY

Editors

C. R. AUSTIN, B.V.Sc., D.Sc.

J. S. PERRY, Ph.D.

56 Illustrations

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PREFACE

FERTILITY has always been a tascinating subject for research, whether viewed academically or clinically, either in an attempt to control or to promote the fertility of the individual or the species. It calls for research from different angles and absorbs the energies of many workers in many disciplines. More recently the urgent necessity for promoting and expanding knowledge in this field has become a pressing international problem. It is therefore most appropriate that the Biological Council should have chosen to devote their Symposium in 1964 to Agents Affecting Fertility and hardly surprising that in view of the wide coverage of the chosen subject, the organizing committee decided to arrange for a review of the field, thus bringing together clinicians, pharmacologists and biologists.

The Symposium was organized by the British Pharmacological Society with the participation of the Nutrition Society, the Biochemical Society, the Physiological Society, the Royal Society of Medicine, the Society of Endocrinology, the Society for the Study of Fertility, the Society of Chemical Industry and the Council for the Investigation of Fertility Control. The members of the Organizing Committee were: A. S. Parkes (Chairman), W. A. Bain, G. W. Harris, H. Jackson, Patricia Scott, G. I. M. Swyer and Eleanor Mears (Secretary). The detailed secretarial work was carried out by Miss Pam Gorden.

The Symposium was held in the Edward Lewis Theatre of the Middlesex Hospital Medical School by permission of the Dean, Sir Brian Windeyer. It was attended by over 400 members of the participating societies and attracted many visitors from overseas. We wish to thank the staff of the Middlesex Hospital Medical School for their help at all stages.

Grateful acknowledgement is made to the Wellcome Trust for

financial support, which enabled us to invite six contributors from the U.S.A., and one each from Israel and France, and to the Ciba Foundation for hospitality afforded to overseas speakers.

ELEANOR MEARS
(Secretary)

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INTRODUCTION

A. S. PARKES

Physiological Laboratory, Cambridge

THIS volume records the proceedings of the eleventh Symposium in the series organized by the Co-ordinating Committee for Symposia on Drug Action, a body sponsored by the Biological Council. The general aim and scope of these Symposia have been fully indicated in the previous volumes of the series. It should be said, however, that these Symposia have made a notable contribution to thought in this field and that the published proceedings have become valuable additions to the literature.

Previous Symposia have dealt with a wide variety of subjects, some being within the field of classical pharmacology and others having a different context. The Symposium on Agents Affecting Fertility ranged from man to insects and from X-irradiation to sex lures. This very wide scope illustrates well the almost ubiquitous importance of the study of fertility in the animal world. Whether in controlling insect invasions or the human population explosion, or in dealing with subfertility in man and domestic animals, the study of agents affecting fertility is a major facet of biology at the present time. In the present Symposium the subject matter was limited in only two ways—we did not deal with the naturally-occurring hormones, and we tried to concentrate not so much on the body of established knowledge as on its active growing points. For that reason, the well known orally-active progestagens which are the active agents in a now well established form of contraception received less attention than their intrinsic importance would demand. Nevertheless because of the social and economic importance of the study of fertility, as

well as its inherent scientific interest, this volume is likely to have a very wide circulation and one not entirely restricted to the scientific world. We have, therefore, avoided the use of the word 'drugs' in the title because the word has, to many, a somewhat unfortunate implication.

This Symposium, like its predecessors in the series, was arranged by an Organizing Committee appointed by the Co-ordinating Committee and supported by a number of societies. A list of members of the Organizing Committee and the names of the supporting societies are given in the Preface. Many of the societies in question are regular supporters of the Symposia in this series, but on this particular occasion they were joined by a number of specialist organizations concerned with the subject of fertility. It should be added that, as in the past, this Symposium was generously supported by the Wellcome Trust and the Ciba Foundation.

As Chairman of the Organizing Committee, it was my very pleasant duty to welcome members of supporting societies and particularly, of course, the invited speakers and other participants from abroad. It would be invidious to mention individuals from such a distinguished company; the contents list of the volume speaks for itself. Rarely have I had the good fortune to see such an eminent and representative gathering of what I ventured at the first session to call 'fertilitarians'. This sobriquet was more than justified by the rapid proliferation of information and ideas which the Symposium engendered and which made the occasion a memorable one for all those who attended.

May 12th 1964. A. S. PARKES

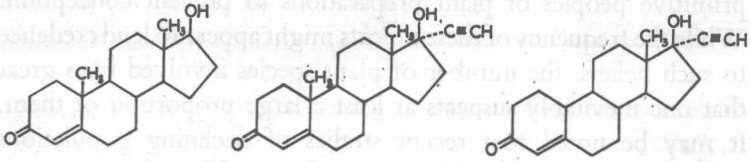
ANTIFERTILITY AGENTS OF PLANT ORIGIN

J. R. PRICE

C.S.I.R.O., Melbourne, Australia

IT is clear from the programme arranged for this Symposium that its major interest hinges around methods of controlling human fertility. This being so, my review of antifertility agents of plant origin must necessarily be brief, since there is little information which will stand up to critical evaluation other than that concerning plant oestrogens. On the other hand, there are a great many reports, published or passed on by word of mouth, of the use by primitive peoples of plant preparations to prevent conception. While the frequency of these reports might appear to lend credence to such beliefs, the number of plant species involved is so great that one inevitably suspects at least a large proportion of them. It may be noted that recent studies of declining populations attribute the cause entirely to the incidence of disease. Depopulation in New Ireland, for example, has been ascribed to malaria and gonorrhoea, and the possibility that contraception may be a contributory factor has been specifically discounted (Scragg, 1957). However, there is no *a priori* reason why there should not be present in plants substances which might be capable of interfering at one point or another with the reproductive cycle and I believe that the need for greater knowledge of oral contraceptives is so urgent that we should investigate these reports, however small the chance of success, rather than risk overlooking potentially valuable information. Unquestionably, the most important approach in searching for substances which meet the various requirements for fertility control is to further our basic scientific knowledge of reproductive physiology. But experience suggests,

where there is a degree of urgency, that almost as important is the kind of empirical approach which has been so successful in providing the medical profession with many—perhaps most—of the battery of drugs it uses today for the treatment of disease—and indeed in providing us with many other kinds of potent biologically active materials, insecticides, herbicides and the like. The screening of culture media from tens of thousands of microorganisms has given us a dozen or so of the antibiotic drugs in common clinical use and it is essentially the same procedure which has made available the numerous useful modifications of the natural steroid hormones. It could scarcely have been predicted that the substitution of an acetylenic group in the 17α-position of the androgenic testosterone molecule (I) would give rise to a substance, ethisterone (II), and by a further step norlutin (III)—with such a dramatic change to high oral progestational activity.

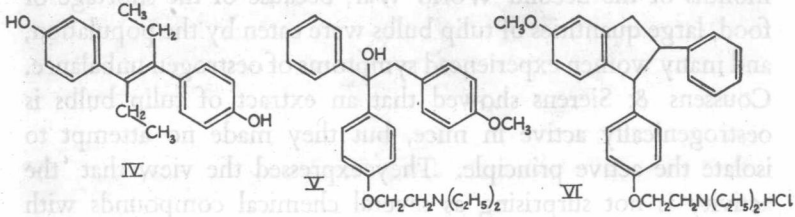


I II III

And despite a superficial structural resemblance to diethylstilboestrol (IV), it is even less likely that one could predict that 1-(*p*-2-diethylaminoethoxyphenyl)-1-phenyl-2-*p*-anisylethanol ((V), MER 25; Segal & Nelson, 1958) and the 2,3-diphenylindenes, such as (VI) (Lednicer *et al.*, 1961), would act as potent antifertility (antioestrogenic) agents.

Thus we must recognize that to have the best chance of speedy success, a search for chemical agents for controlling fertility should embrace empirical as well as more fundamental studies. I have already referred to one empirical procedure which has produced valuable information—the screening of culture media for antibiotic activity. This is, in effect, the screening of a complex

mixture of substances and as such is little more than a refinement of the time-honoured procedure by which man, over many thousands of years, has searched for and found those drugs made use of in so-called 'folk-medicine'. It is well known that in the majority of instances the beneficial effects ascribed to folk-medicine are found to be without foundation when subjected to critical experimental examination. But it is also true that from a small proportion of these drugs have been derived agents such as morphine, reserpine, quinine, ephedrine, mescaline, santonin and others which have proved of real value, many of them being still in use. What is more important is that these and others have given



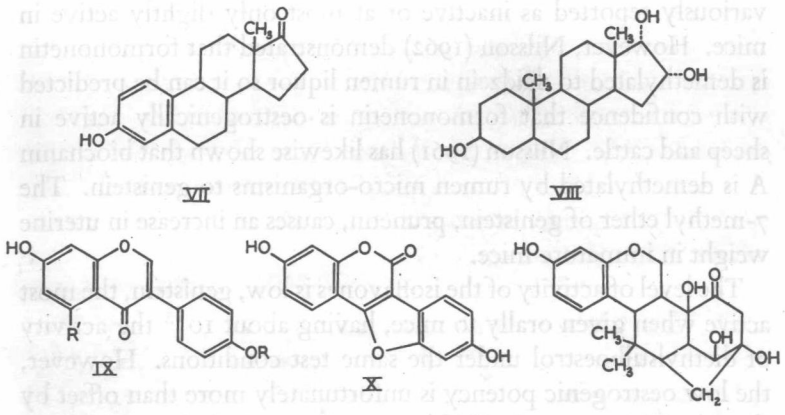
rise to better drugs by providing a pattern on which the organic chemist can ring the changes. Moreover, he can often do this with sufficient versatility to produce cheaper or more effective substances, as exemplified by the synthetic cocaine analogues. Granted, then, that fundamental studies of the reproductive system should be supplemented by an empirical approach to fertility control, how should such empirical programmes be orientated? Has the plant kingdom anything to offer as a starting point for the possible development of useful antifertility agents? On the face of it, it has a great deal to offer, and it is my task, this morning, to present a picture of the situation which will enable you to form an opinion as to the amount of effort which should be put into this area as compared with others. The following account of what is known of antifertility agents of plant origin is selective rather than comprehensive; no consideration has been given to abortifacients.

Oestrogenically active substances

There are known to be present in certain plants substances with marked antifertility effects on grazing animals. This subject has been reviewed recently by Moule, Braden & Lamond (1963). In an earlier review, not restricted to problems of animal production, Bradbury & White (1954) listed over 50 species of plants reported to exhibit oestrogenic activity. I propose to comment only on those in which the active principle has been identified, with one interesting exception, the bulb of the garden tulip. It was reported by Coussens & Sierens (1949) that during the last months of the Second World War, because of the shortage of food, large quantities of tulip bulbs were eaten by the population, and many women experienced symptoms of oestrogen unbalance. Coussens & Sierens showed that an extract of tulip bulbs is oestrogenically active in mice, but they made no attempt to isolate the active principle. They expressed the view that 'the activity is not surprising as several chemical compounds with steroid structure are found in this plant material'. This may well be true (though it does not appear to be substantiated by the literature, see for example, Hegnauer, 1963) since steroidal substances have been found in a great many plant species. In this context the most significant report is that of the isolation and identification of oestrone (VII) by Butenandt & Jacobi (1933) from palm kernel press-cake (*Elaeis guineensis*). Other claims concerning the isolation of steroidal oestrogens from plants lack the necessary supporting chemical data, but the occurrence of plant constituents closely resembling the animal hormones is confirmed by a recent well-authenticated identification, namely the isolation of 5α-androstan-3β, 16α, 17α-striol from *Aplopappus heterophyllus*, a plant which is responsible for a disease known as 'milk sickness' in the higher animals (Zalkow, Burke & Keen, 1964). Bednar & Zenisek (1961) have reported recently that hop extracts are oestrogenic (see also Chury, 1960) and that the

β-acids (lupulone, colupulone, adlupulone) are partially responsible for this activity; the α-acids are inactive.

By far the most important manifestation of oestrogenic activity in plants is due to isoflavones and related compounds. Infertility in sheep resulting from the ingestion of these substances was first recognized in Western Australia during the Second World War when in certain areas lamb marking percentages fell from about 80% to as low, in some instances, as 10%. Generally referred to nowadays as 'clover disease', the condition has been recognized



in other areas of southern Australia, and in eastern Australia, New Zealand and the United States. Infertility of cattle due to ingestion of oestrogenic pasture has also been observed in many countries including Israel, Italy, Germany, the United States and Australia. Because of their economic implications, pasture oestrogens are currently being subjected to intensive investigation. As already stated, the causative agents are isoflavones or substances structurally related to them, notably the coumaranocoumarin coumestrol.

Isolated occurrences of isoflavones and their derivatives have been reported from several plant families such as the Iridaceae, Moraceae, Rosaceae, Compositae and Coniferae, but the major source is the

sub-family Papilionatae of the Leguminosae in which they have so far been reported from 34 genera. A number of these genera, such as *Trifolium* and *Medicago*, include valuable pasture species, while others may be grazed by stock living on unimproved pastures. The isoflavones which have been shown to exhibit oestrogenic activity in experimental animals are genistein (IX; $R = H$, $R' = OH$), its 4′-methyl ether biochanin A (IX; $R = CH_3$, $R' = OH$), and daidzein (IX; $R = R' = H$). Formononetin (IX; $R = CH_3$, $R' = H$), the 4′-methyl ether of daidzein has been variously reported as inactive or at most only slightly active in mice. However, Nilsson (1962) demonstrated that formononetin is demethylated to daidzein in rumen liquor so it can be predicted with confidence that formononetin is oestrogenically active in sheep and cattle. Nilsson (1961) has likewise shown that biochanin A is demethylated by rumen micro-organisms to genistein. The 7-methyl ether of genistein, prunetin, causes an increase in uterine weight in immature mice.

The level of activity of the isoflavones is low, genistein, the most active when given orally to mice, having about 10^{-5} the activity of diethylstilboestrol under the same test conditions. However, the low oestrogenic potency is unfortunately more than offset by the amounts present, in *Trifolium* species particularly, which may be such that a sheep grazing a clover-predominant pasture might ingest up to 10 to 15 g isoflavone per day. Moreover, there is in many clovers a small amount of the coumaranocoumarin coumestrol (X) (and probably analogues of it) which is about 30 times as active as genistein when given orally to mice. Genistein and coumestrol have been shown to be what Emmens (1941) describes as pro-oestrogens, they are believed to be converted in the animal body into the true, oestrogenically-active material.

The most potent substance of this group, miroestrol (XI), is not involved in animal infertility problems but was isolated as a result of a report that the roots of a plant, now named *Pueraria mirifica*, were used in northern Thailand as a rejuvenating drug. The

structure of miroestrol is rather more complex than that of the simple isoflavones but it is clearly biosynthetically related to them. In this connexion, daidzein has been reported present in the roots of three other *Pueraria* species (Shibata, Murakami & Nishikawa, 1959). The oestrogenic activity of miroestrol is very much greater than that of the isoflavones, being comparable with that of oestradiol; miroestrol is more than three times as active as diethylstilboestrol in causing an increase in uterine weight in the immature mouse when given orally.

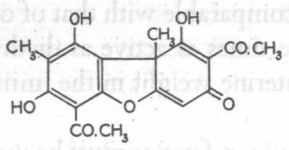
Before concluding this brief account, reference must be made to the possible presence in plants of oestrogen inhibitors. For example, Adler (1962) claims to have separated and concentrated an oestrogen-inhibiting substance from *Medicago sativa*, which not only inhibits the action of the oestrogens in that species, but also inhibits the action of oestradiol and diethylstilboestrol. Further work in this field will be awaited with interest.

Other substances of known chemical constitution

In this section I shall refer briefly to the few substances, other than oestrogens, which are derived from plants and are of known structure, for which antifertility activity of any kind has been reported. Firstly, certain plant alkaloids, which are unlikely to be of significance in relation to human fertility, have been shown to reduce the fertility of experimental animals. These alkaloids, listed by Jöchle (1962), include reserpine and morphine—stated to inhibit ovulation—and pilocarpine, physostigmine, certain of the ergot alkaloids and (if it may be classified as an alkaloid) the growth hormone, indolyl-3-acetic acid, which inhibits nidation. Jöchle suggests that it might be possible to develop a suitable morphine analogue which is non-addicting and free of other side-effects. One other alkaloid, the well-known antimitotic agent colchicine, and certain of its derivatives, have been studied by several workers and shown to interrupt pregnancy. For what it may be worth, the liliaceous species *Gloriosa superba* which contains colchicine, has a

reputation in Indonesia as an abortifacient (van Steenis-Kruseman, 1953).

Other substances exhibiting antimitotic activity are the lichen colouring matter usnic acid (XII) and the lignanes podophyllotoxin



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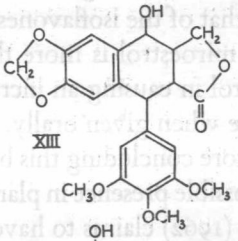

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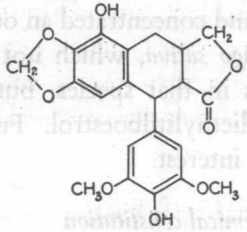
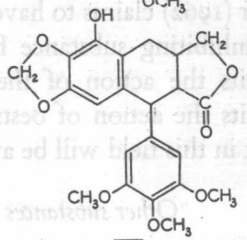


XIV



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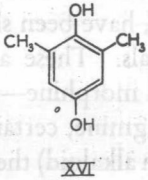
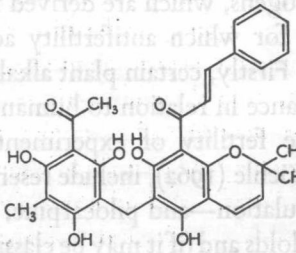


XVI



XVII

(XIII), α-peltatin (XIV) and β-peltatin (XV). The effects of these substances on the fertility of mice have been reported by Wiesner & Yudkin (1955), Wiesner, Wolfe & Yudkin (1958), and others. Administration of usnic acid by stomach tube to mice before pairing or immediately after copulation was usually not followed