ON DOISYNOLIC ACIDS, A NEW CLASS OF ESTROGENS

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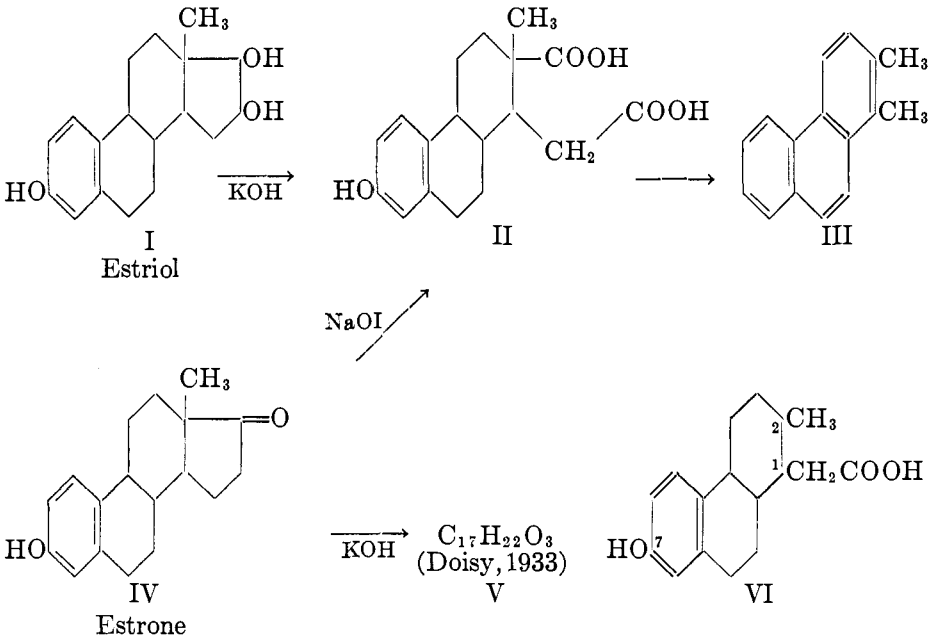
CONTENTS

I. MONO- AND DI-CARBOXYLIC ACIDS FROM ESTROGENIC HORMONES

So far, five estrogens have been found in nature—estrone, estradiol, estriol, equilenin, and equilin—and in addition numerous synthetic ones have been prepared, which are all more or less related to stilbestrol. During the past few years we investigated a group of substances which are very closely related to the natural estrogens, and thus have collected new and interesting information on the hormone specificity problem.



In 1932 Marrian (22) succeeded in opening the five-membered ring of estriol (I) by fusion with potassium hydroxide and obtained a dicarboxylic acid which we have named "marrianolic acid" (II). Butenandt (8) converted this acid into 1,2-dimethylphenanthrol by dehydrogenation and further into 1,2-dimethylphenanthrene (III) by distillation with zinc dust, thus confirming the presumed constitution of the estrogenic hormones.

Much less attention was paid to a monocarboxylic acid (V), melting at 195°C., which was obtained by Doisy and collaborators (21) in 1933 by melting estrone (IV) with potassium hydroxide and which they believed to have the formula $C_{17}H_{22}O_3$. The American investigators first claimed that the new carboxylic acid showed several times the estrogenic activity of the starting hormone and that it was active even when given orally. However, they soon recalled this statement.

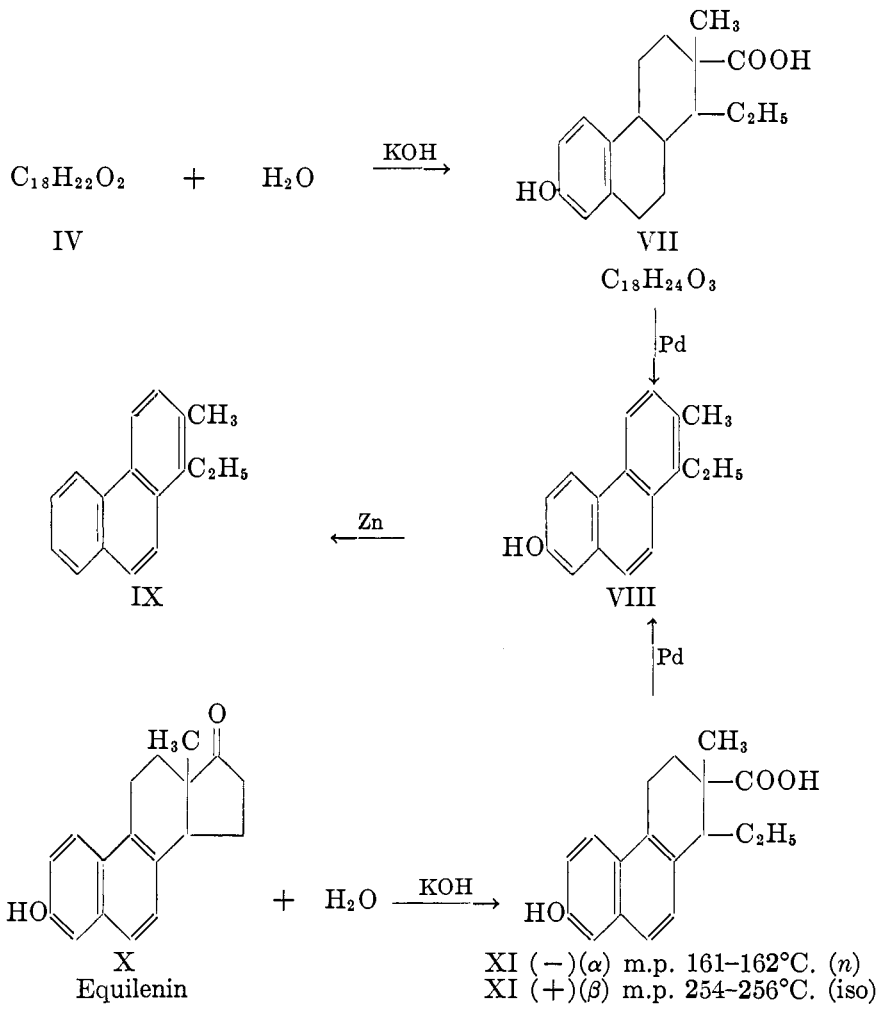
In patent applications of the Schering Company (30) dated 1937 and 1939 the question of the potassium hydroxide melt of estrogenic hormones was again taken up by Hohlweg and Inhoffen, and it was ascertained that estradiol yielded the same monocarboxylic acid as estrone. Analogous acids were also obtained from equilenin and equilin, although in a rather crude state. It is remarkable that these acids were active in rats when administered orally in doses of 1–6 γ. According to the patents the monocarboxylic acids belonged to the C_{17} series, and the acid from estrone was assigned the constitution of a 2-methyl-7-hydroxy-octahydrophenanthrene-1-acetic acid (VI).

II. ELUCIDATION OF THE CONSTITUTION OF THE MONOCARBOXYLIC ACIDS (25)

To us, the proposed formulation did not appear very likely and we decided to reinvestigate the carboxylic acids obtained from estrogenic hormones. We first prepared marrianolic acid (II) (14) and bisdehydromarrianolic acid (12) from estrone and equilenin by oxidation with hypoiodite. A study of their reactions simplified the elucidation of the corresponding monocarboxylic acids.

The first substance that we investigated in the monocarboxylic acid series was the acid from estrone (or estradiol) originally found by Doisy and which we have called "doisynolic acid" in honor of him. The fact that its ester was difficult to saponify spoke against its formulation as a hydrophenanthreneacetic acid (VI), as suggested in the German patents. Its constitution was ascertained by dehydrogenation with palladium to 1-ethyl-2-methyl-7-phenanthrol (VIII) and then by dehydroxylation with zinc to 1-ethyl-2-methylphenanthrene (IX), the latter having previously been synthesized by Haworth (11). Therefore, the formula $C_{18}H_{24}O_3$ corresponded to doisynolic acid (VII) and its formation occurred without loss of carbon by a simple hydrolytic fusion of the five-membered ring. In the case of estradiol preliminary dehydrogenation must have taken place. Similar reactions in the camphor series have been known for a long time (9, 10).

Whereas the doisynolic acid was thus obtained in a relatively simple manner in a yield of about 50 per cent, fusion of equilenin (X) with potassium hydroxide yielded a complex mixture of acids. From this mixture we were able to isolate,



although in a poor yield, the two optically active acids α and β, owing to a difference in their acidity. Both could be dehydrogenated to the same 1-ethyl-2-methyl-7-phenanthrol (VIII) as was obtained from doisynolic acid. The two optically active acids, the α- and β-bisdehydrodoisynolic acids, were therefore diastereoisomers of the formula XI, and contained two centers of asymmetry.

Whereas no estrogenic activity can be attributed to the dicarboxylic acids, the oral threshold value of doisynolic acid in castrated female rats is 1.5 γ (compare table 1). But it is surpassed considerably by the (−)-α-bisdehydrodoisynolic acid with a threshold value of 0.05–0.07 γ, which is at least 20 times as effective as the crude acids obtained by Hohlweg and Inhoffen from equilenin. The isomeric β-acid is inactive.

We first assumed that the biologically active α-acid corresponded sterically to estrone and equilenin, and therefore designated it as the normal acid and the

β-derivative as the iso acid. However, bisdehydromarrianolic acid possesses a dextrorotation, as do also equilenin, estrone, estradiol, and doisynolic acid, whereas the active α-acid is levorotatory. This will be discussed again later on in this review. It must be that during the splitting of equilenin with potassium hydroxide, a partial isomerization occurs at one of the two centers of asymmetry, C_1 or C_2, which may be attributed to the greater lability of the more strongly aromatized nucleus, since it does not occur with estrone.

III. THE SYNTHESIS OF BISDEHYDRODOISYNOLIC ACID

The elucidation of the constitution of the doisynolic acids enabled us to accomplish the total synthesis of the bisdehydro derivatives. The starting point was Cleve's acid (XII) which, according to Butenandt (7), can be transformed through a series of intermediates to β-bromoethylnerolin (XIII) and thence into 1-keto-7-methoxytetrahydrophenanthrene (XIV). Bachmann (4) in 1939 obtained from the latter 1-keto-2-methyl-7-methoxytetrahydrophenanthrene-2-carboxylic acid ester (XV). It was used by him in carrying out his elegant equilenin synthesis, which is up to now the only total synthesis of an active steroid hormone.

By reacting ethylmagnesium bromide with XV and dehydrating the resulting tertiary carbinol the ethylidene derivative (XVI) was obtained. After reduction of the double bond and demethylation of the ester and the ether groups there resulted a mixture of the two racemic bisdehydrodoisynolic acids XVIIα and XVIIβ (13).

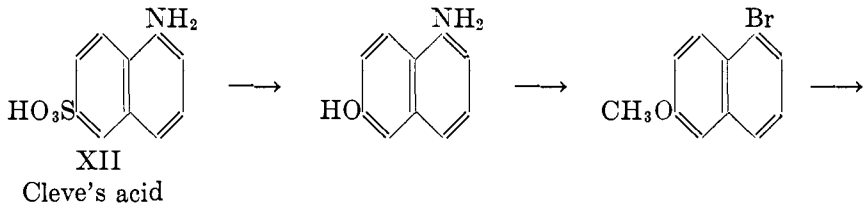
The lower-melting α-racemate (normal) proved highly active in rats, whereas the higher-melting β-racemate (iso) was ineffective. Finally, we also succeeded in separating the α-racemate into the two antipodes *via* the menthol ester (29). The levorotatory derivative was identical with the α-acid (XIα) from equilenin. The total activity of the racemate can be ascribed to this antipode.

We were able to shorten the synthesis of the acids XVII (1) from twelve steps to four steps by reacting the sodium salt of the enol of propionylpropionic acid ester with β-bromoethylnerolin and cyclizing with sulfuric acid the keto ester (XVIII) thus obtained. It is possible to carry out the hydrogenation of the ethylidene compound (XVI) in such a way that mainly the α-acid is produced.

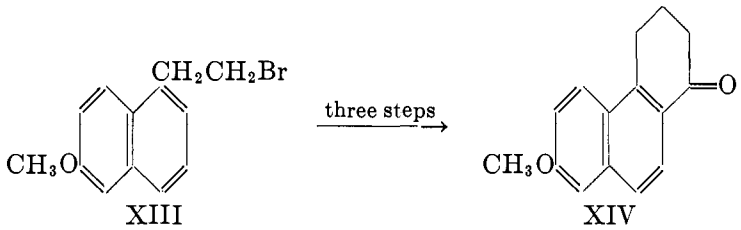
IV. THE ACTIVITY OF THE DOISYNOLIC ACIDS

In table 1 the threshold values (32, 33) of the doisynolic acids in the estrus test on castrated rats are compared with the estrogenic hormones and diethylstilbestrol. The acids were dissolved in aqueous bicarbonate and were applied in either single or subdivided doses.

The (−)-α-bisdehydrodoisynolic acid shows the highest activity, the racemate being half as active as the levo-form. It is interesting that the activity of the 7-methyl ethers does not differ much from that of the free α-bisdehydrodoisynolic acids. Under the name "Fenocylin" the methyl ether of the α-acid has lately been submitted for clinical trials. It is interesting that the methyl ethers of some of the natural hormones exhibit a markedly lower activity: thus, the activity of estradiol methyl ether is only about 1/30th that of the free hormone.



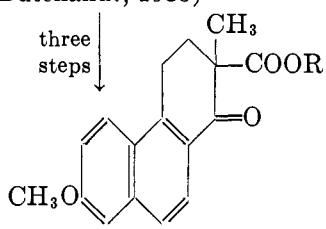
XII
Cleve's acid



XIII
β-Bromoethylnerolin

XIV
(Butenandt, 1935)

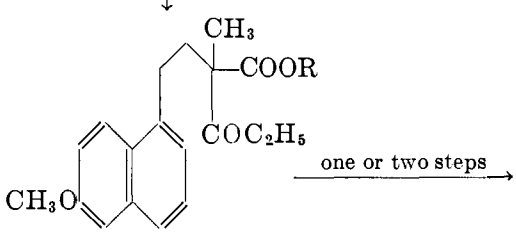
three steps



XV
(Bachmann, 1939)

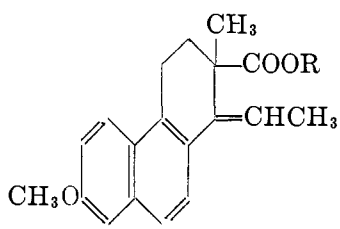
one step

two steps



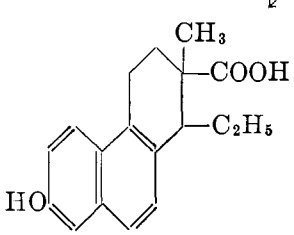
XVIII

one or two steps



XVI

two steps



XVII (racemic)(α) m.p. 204°C. (*n*)
XVII (racemic)(β) m.p. 235–238°C. (iso)

While the activity of the estrogenic hormones and diethylstilbestrol depends to a great extent on their mode of administration, the uniformly high activity of α-bisdehydrodoisynolic acid and its 7-methyl ether in rats is remarkable.

TABLE 1

Estrus test on castrated rats

ESTROGENS	SUBCUTANEOUS ADMINISTRATION		ORAL ADMINISTRATION
	Single dose	Five divided doses	Single dose
	Threshold values in γ		
α-Bisdehydrodoisynolic acid* (−)	0.05–0.07	—	0.05–0.07
7-Methyl ether* (−)	0.05–0.07	—	0.05–0.07
α-Bisdehydrodoisynolic acid* (racemic)	0.1–0.15	0.1–0.15	0.1–0.15
7-Methyl ether* (racemic)	0.1–0.15	0.1–0.2	0.1–0.15
β-Bisdehydrodoisynolic acid* (+)	>100	—	>100
Doisynolic acid (from estrone)* (+)	0.7–1.0	0.7–1.0	1.5
Estrone†	10–15	0.5–1.0	20–30
Estradiol†	1–2	0.3	20–30
Equilenin†	100–150	5–15	300–500
Diethylstilbestrol†	2–3	0.3–0.4	2–3

* As sodium salt, in water.

† In water.

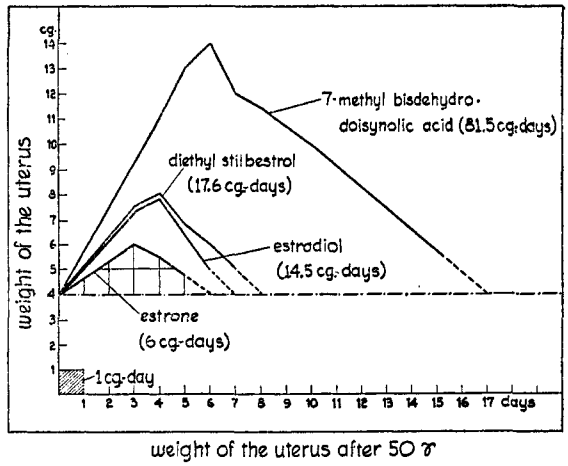


FIG. 1. Change in weight of uterus of castrated rats after administration of estrogens

The increase and decrease of the weight of the uterus of castrated rats after oral administration of a single dose of 50 γ of four different estrogens is shown in figure 1. The total activity is characterized by the area between the respective curves and a horizontal line corresponding to the initial weight of the uterus; this is the so-called "output of activity" and can be measured in cg.-days. Thus, 1 cg.-day means an increase of weight of 1 cg. maintained during 1 day. This

new biological unit, which we introduced earlier, is independent of the scale chosen and when properly adapted can be advantageously used to solve many similar problems (24, 27).

The high oral activity of Fenocylin in rats enabled Meier and Tschopp (23) to determine its concentration in various organs after a single administration of 1 mg. per kilogram. A large series of animals was treated, and they were sacrificed groupwise after various intervals. The concentration in different organs was determined by feeding them to castrated rats. Table 2 shows the content of the organs after 1, 6, and 10 days. It is striking that the highest concentration was attained in the intestines, about half as much in the liver, and only about one-sixth in the uterus. This was true even when Fenocylin was given subcutaneously. Thus it was shown that the sensitiveness of the receptor organ was more important than the concentration of the drug. It is very interesting

TABLE 2

Concentration of methylbisdehydrodoisynolic acid (Fenocylin) in the organs of female rats in gamma per cent, after single doses of 1 mg./kg. by gastric route

ORGAN	AFTER		
	1 day	6 days	10 days
Intestines	400	27	5
Liver	130	13	2.5
Uterus	57	5–6	<1
Heart and muscles	27	2.5	1–2
Adipose tissues	13	2.2	<1
Skin	10	1	<1
Spleen	8	<1	<1
Brain	2.5	<1	<1

that a similar finding was recently reported by LeBlond (20). He found that after the injection of thyroxine containing radioactive iodine the main portion of the substance was located in the intestine and only about half this amount in the liver. Perhaps other biologically active acids behave in a similar manner.

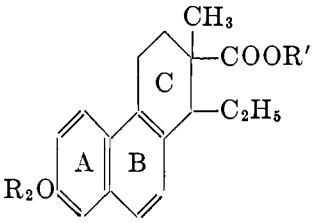
The concentration of Fenocylin in the organs of the rat quickly decreased during the first days. After 10 days only traces were detected, whereas full estrus continued much beyond that time.

Although the concentration of Fenocylin in the uterus constantly decreased, the weight of the uterus increased from 50 to 290 mg. up to the sixth day, when the amount (5–6 γ per cent) in the uterus was only about 10 per cent of the initial value. As the Fenocylin concentration fell still further, the relatively high uterus weight decreased and dropped to 170 mg. at the tenth day.

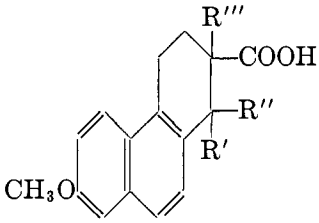
V. DERIVATIVES, HOMOLOGUES, AND ANALOGUES OF α-BISDEHYDRODOISYNOLIC ACID (26)

The total synthesis of bisdehydrodoisynolic acid gave rise to the preparation of numerous derivatives, homologues, and analogues; however, only the biological results will be discussed here.

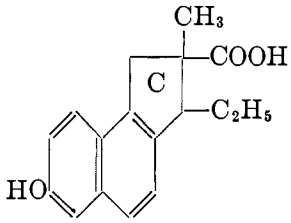
Regarding the ethers and esters (XIX) of the α-bisdehydrodoisynolic acid it



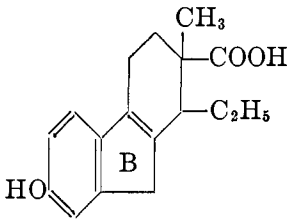
XIX



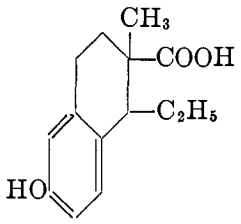
XX



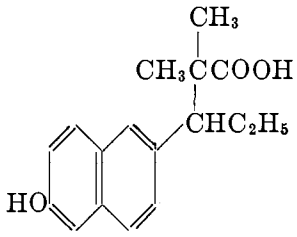
XXI



XXII



XXIIIα and XXIIIβ



XXIV

might be mentioned that the higher esters, especially the 7-benzoate and the 7-butyrate of the methyl ester of the α-acid, showed the well-known ester effect as found with the natural hormones, i.e., prolonged action after injection of an oily solution.

The derivatives with hydrogen or different alkyl groups at C_1 or C_2 (XX) were more interesting. In part, special methods had to be developed for their preparation. Acids without alkyl groups or with only one methyl group at C_1 or C_2 were completely or practically inactive; with two to three methyl groups or one ethyl and one methyl group at C_1 and C_2 the potency reached a maximum value with 0.1–0.2 γ. Further elongation of the side chains, however, resulted in a loss of activity. If the alkyl groups at C_1 or C_2 were closed to a five- or six-membered ring the activity disappeared. Replacement of the six-membered ring C by a five-membered ring to give the C-nor acid (XXI) had the same effect. In contrast to this, the B-normonodehydrodoisynolic acid (XXII) proved to be active, with a threshold value of approximately 1 γ.

Neither of the two diastereoisomers of the 1-ethyl-2-methyl-6-hydroxy-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (XXIIIα, XXIIIβ), which may be considered to be a simplified model of doisynolic acid, showed any activity. One of these isomers was also prepared by A. Horeau (17) in Paris according to our first bisdehydrodoisynolic acid synthesis.

Recently Horeau (18) has synthesized "bisdehydrodoisynolic" acids with an opened ring C. These acids belong to the naphthyl-β-propionic acid series and show estrogenic activity. He called them "allenolic acids" in honour of Allen. In figure 2 is shown the activity of the most active compound of this group, the α,α-dimethyl-β-ethyl derivative (XXIV), on castrated rats in comparison with the racemic 7-methyl ether of α-bisdehydrodoisynolic acid and diethylstil-

bestrol. Comparing the data for a 50 per cent response of the animals, the new acid is about one-seventh as active in the rat as Fenocylin.

It was especially interesting to substitute the carboxyl group of the α-bisdehydrodoisynolic acid by other radicals. Replacement by —CH_2COOH (XXV), —$COCH_3$ (XXVI), or —$COCH_2OCOCH_3$ (XXVII) destroyed the activity, but the aldehyde (XXVIII) and the primary carbinol (XXIX), with threshold values of 0.3–0.5 γ, were surprisingly only slightly less active than the carboxylic acid (0.1 γ). In fact, they showed a more prolonged action after subcutaneous administration. Even when the carboxyl group was replaced by a methyl group

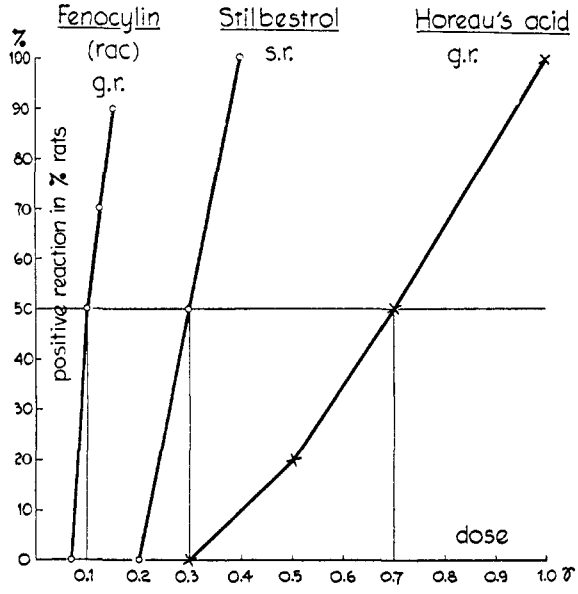
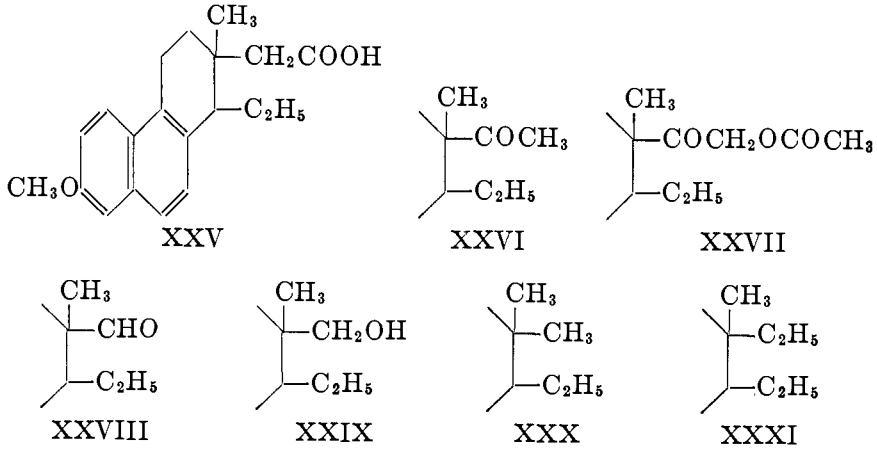


FIG. 2. Estrus test in castrated rats, each dose being applied once to ten animals. g.r = gastric route; s.r. = subcutaneous route.



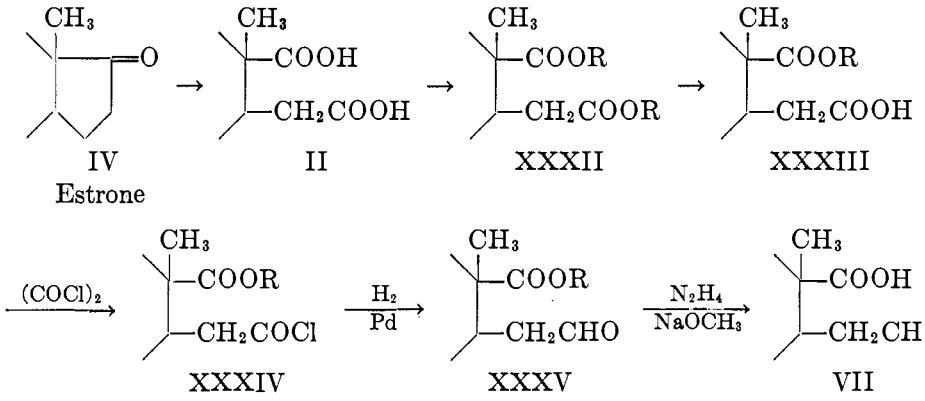
(XXX) the threshold value still attained 5–20 γ. However, the ethyl derivative (XXXI) was inactive.

The fact that the carboxylic acid and the corresponding carbinol show about the same physiological activity has also been observed in connection with vitamin A, with pantothenic acid, and perhaps with oxybiotin. Probably oxidation of the carbinol or aldehyde group occurs in the organism.

VI. STERIC RELATIONSHIP BETWEEN ESTROGENIC HORMONES AND THE DOISYNOLIC ACIDS (15)

As has already been shown, two diastereoisomeric bisdehydrodoisynolic acids are formed from equilenin by fusion with potassium hydroxide, the levorotatory "normal" or α-acid, and the dextrorotatory "iso" or β-acid. Since fusion with potassium hydroxide is a very drastic reaction which obviously is responsible for the partial rearrangement of the substituent at C_1, we searched for a more unequivocal series of reactions.

Such a one is the stepwise transformation of estrone (IV) *via* marrianolic acid

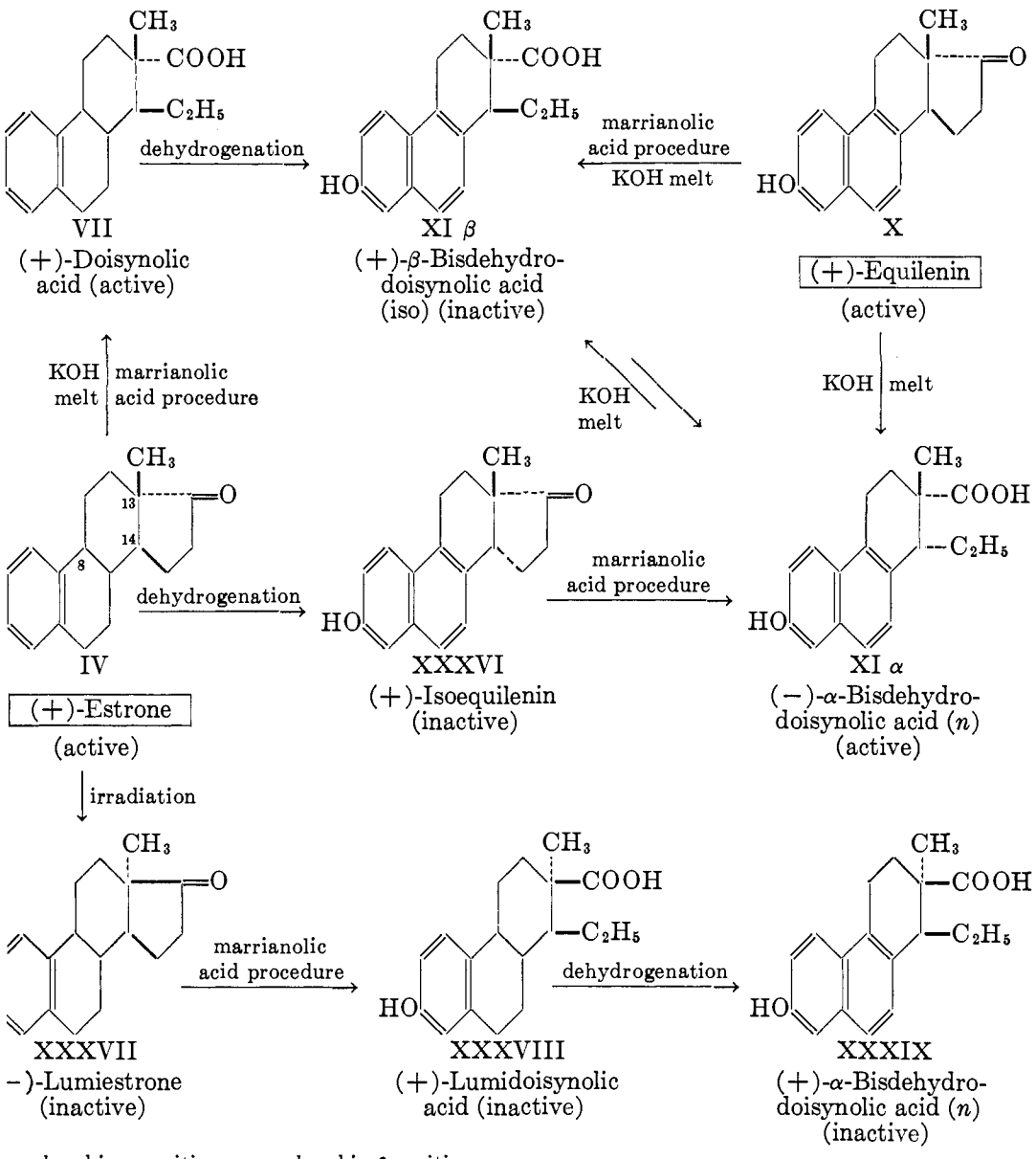


(II) and the intermediates XXXII to XXXV to the same dextrorotatory doisynolic acid (VII) as was obtained by the alkali hydroxide fusion of estrone. However, with the same procedure the active (+)-equilenin (X) led to the inactive (+)-β-bisdehydrodoisynolic acid (iso) (XIβ) and the inactive (+)-isoequilenin (XXXVI) to the active (−)-α-bisdehydrodoisynolic acid (normal) (XIα). In the latter case the change in rotation occurs during the formation of the respective bisdehydromarrianolic acid, obviously without change of configuration.

By irradiation of estrone (IV) rearrangement occurs at C_{13}, leading to lumiestrone (XXXVII). This was transformed by the marrianolic acid procedure to (+)-lumidoisynolic acid (XXVIII), which on dehydrogenation gave the dextrorotatory inactive antipode of α-bisdehydrodoisynolic acid (XXXIX).

The reduction of the monoaldehydes, which in the marrianolic acid procedure are obtained as intermediate products, with hydrazine hydrate in the presence of sodium methoxide at 190°C. by the method of Wolff–Kishner does not, however, quite exclude rearrangements. For example, we have found that the α- and β-

bisdehydrodoisynolic acids may be converted into each other to a certain extent by heating with aqueous alkali hydroxide.



= bond in α-position; — = bond in β-position.

Recently we were able to confirm our former result by boiling the mercaptal of the (+)-β-bisdehydromarrianolic monoaldehyde with nickel in alcoholic solution. The steric relationship was further corroborated by mild dehydrogenation

of (+)-doisynolic acid (VII) in the form of its ether-ester to the inactive (+)-β-bisdehydrodoisynolic acid derivative (XIβ). Previously Butenandt (6) had obtained the (+)-isoequilenin (XXXVI) from estrone (IV) in a similar manner, but in this case inversion took place at C_{14}, in the α-position to the naphthalene ring.

Provided that rings C and D are *trans* in both estrone and equilenin, as is assumed in our formulas, it seems now well established that in doisynolic acid the carboxyl and ethyl groups are correspondingly in the *trans* position, while in the highly active (−)-α-bisdehydrodoisynolic acid they are in the "unnatural" *cis* position.

VII. "DOISYNOLIC ACIDS" DERIVED FROM ANDROGENS (16)

In attempting to answer the question as to whether there also exist any "doisynolic acids" which exhibit androgenic activity, a similar change of androgenic hormones suggested itself. Direct fusion with potassium hydroxide was out of the question in this case, but the stepwise transformation *via* the dicarboxylic acids led to the desired compounds.

Starting from dehydroisoandrosterone (XL), our procedure is illustrated by formulas XLI to XLV.

The intermediate "marrianolic acid" (XLI) (34) is related to the etiobilienic acid of Wieland. The new, purely hydroaromatic "doisynolic acids" (XLII to XLV) may be considered as analogues of dehydroandrosterone, of androstenedione, or of testosterone, as well as of isoandrosterone and androstanedione. Unfortunately, they were completely inactive in androgenic and estrogenic tests.

VIII. MONODEHYDRODOISYNOLIC ACIDS (2)

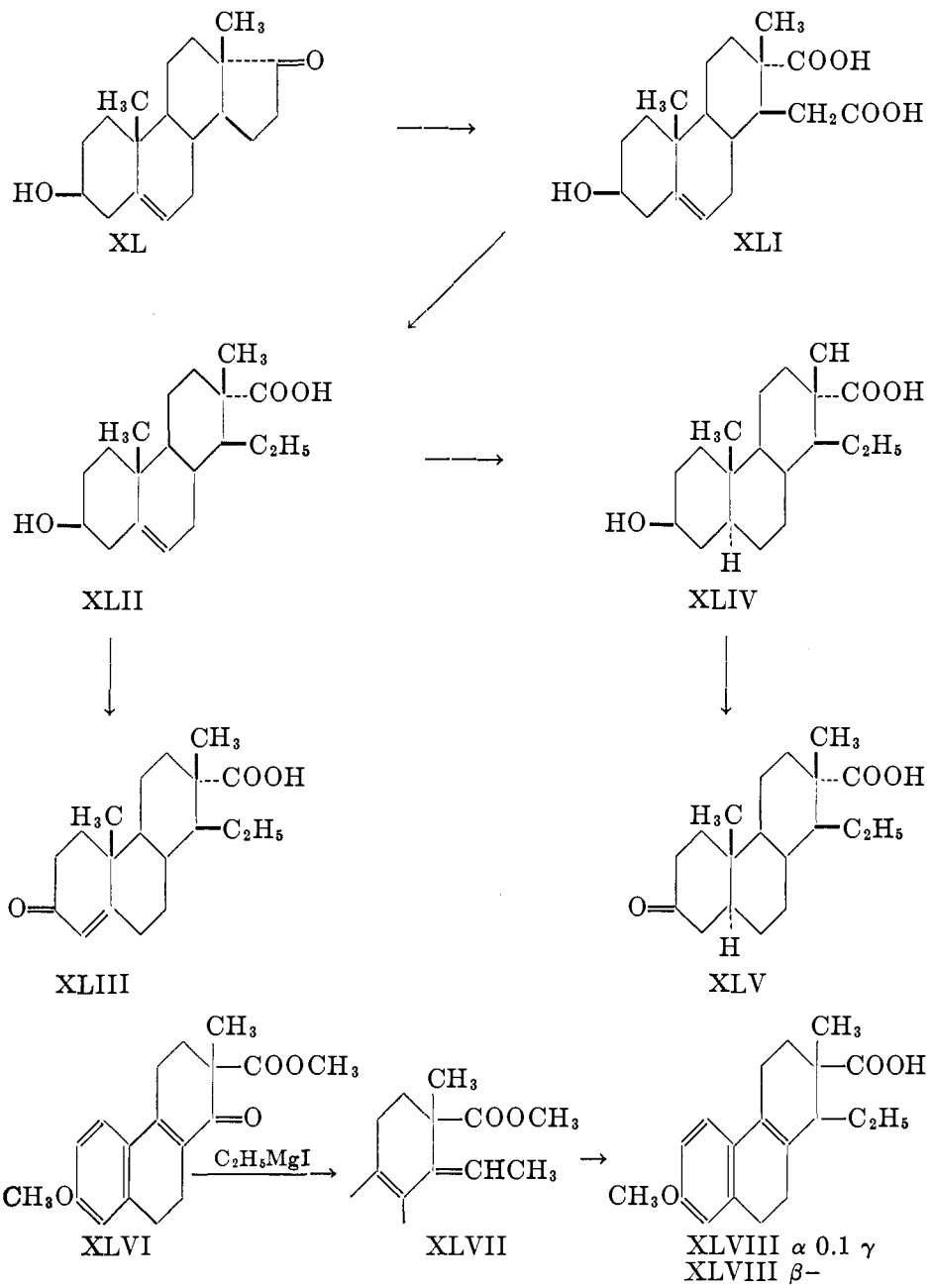
We also were interested in the total synthesis of those doisynolic acids which are di- or tetra-hydrogenated in ring B. The preparation of monodehydrodoisynolic acids with a ditertiary double linkage did not present particular difficulties, as the number of possible isomers is not greater than in the case of the bisdehydrodoisynolic acids. However, we found that the partially hydrogenated ring had a great tendency to turn into the purely aromatic ring.

We started our synthesis with the hexahydrophenanthrene-β-ketocarboxylic acid ester (XLVI), already obtained by Bachmann and coworkers (5) in 1942 in their attempt to prepare natural estrone. By proceeding *via* the ethylidene derivative (XLVII) we obtained two diastereoisomeric 7-methylmonodehydrodoisynolic acids (XLVIIIα) and (XLVIIIβ), of which the lower-melting α-acid had an oral threshold value in rats of 0.1 γ. It proved to be as effective as the racemic α-bisdehydrodoisynolic acid.

Sterically the highly active α-monodehydrodoisynolic acid corresponds to the equally active α-bisdehydrodoisynolic acid into which it can be converted by dehydrogenation of its ether-ester with palladium.

IX. TOTAL SYNTHESIS OF DOISYNOLIC ACIDS (3)

The doisynolic acid from estrone contains four centers of asymmetry. Sixteen isomers and eight racemates are possible. As was to be expected, undesirable

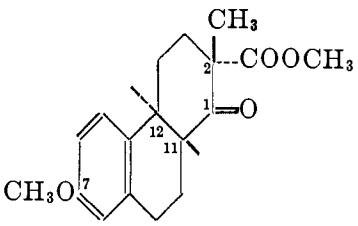


mixtures were formed by the catalytic hydrogenation of the mono- and bis-dehydrodoisynolic acids; this also follows from similar experiments of the American workers Hunter and Hogg (19).

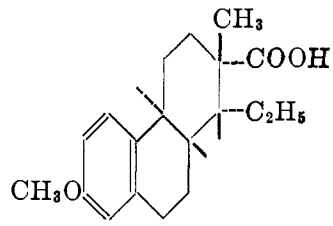
Owing to a method recently recommended by R. Robinson, the action of sodium on the 7-methylether-2-methylester-α-monodehydrodoisynolic acid (ester

*Keto esters**
XLIX

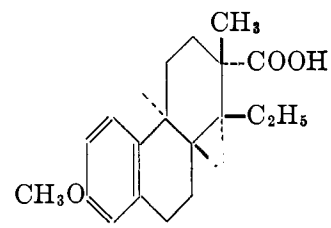
*Doisynolic acids**
L



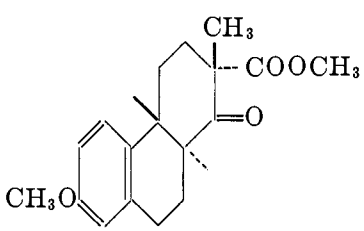
(A) m. p. 133–135°C.
(101–102°C.)



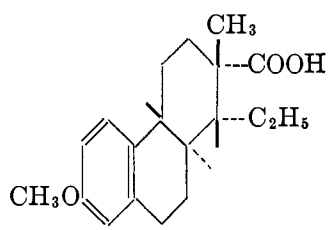
(0.05 γ)
(Aα) m. p. 187–188°C.



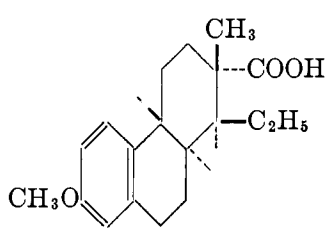
(3 γ)
(Aβ) m.p. 227–229°C.



(B) m.p. 127–128°C.

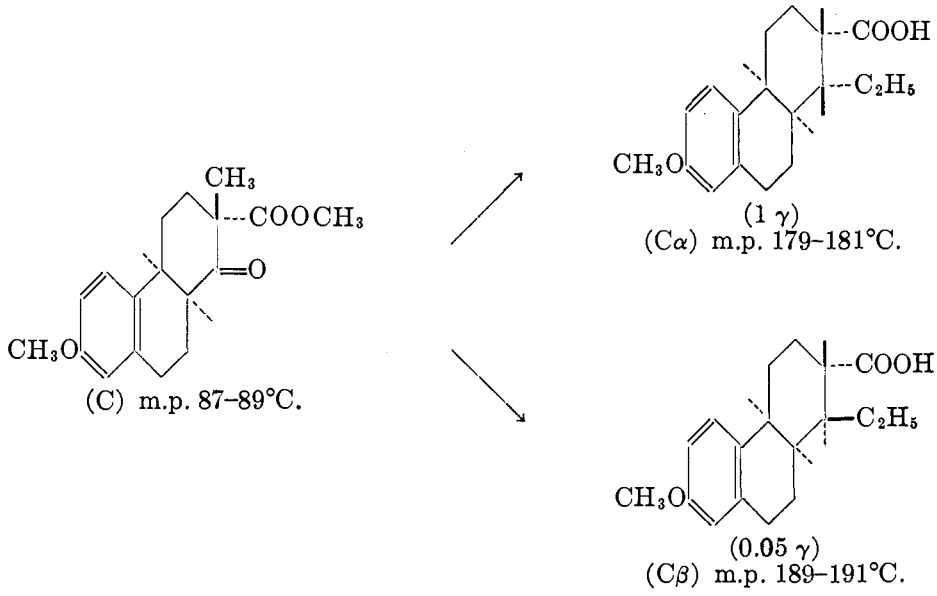


(100 γ)
(Bα) m.p. 213–215°C.



?
(Bβ)

*The racemic methyl ethers are characterized here by the formula of only one of the enantiomers. To each formula therefore is to be added its antipode. The melting points (in °C.) and the threshold values (by gastric route) are those of the racemates.



of XLVIIIα) in a mixture of alcohol and liquid ammonia has enabled us to reduce the ditertiary double bond in such a manner that, after saponification of the reaction mixture, two racemic 7-methyldoisynolic acids of melting point 187–188°C. and 213–215°C., respectively, were obtained in a crystalline state, although in a poor yield. The lower-melting racemic acid with a stomachal threshold value of 0.05 γ proved to be about 30 times more effective than the "natural" (+)-doisynolic acid from estrone. The higher-melting one, however, was almost inactive.

Robinson and Walker (28) in 1936, and later Bachmann and collaborators (5) in their attempt to obtain natural estrone by total synthesis, had already prepared 1-keto-2-methyl-7-methoxyoctahydrophenanthrene-2-carboxylic esters (XLIX). They possess three centers of asymmetry, and accordingly four racemates are possible. The British as well as the American research workers obtained the keto esters only as oily mixtures. We have been able to isolate three racemic methyl esters A, B, and C (XLIX) of the four possible ones in a pure crystalline state. A fourth crystallizate proved to be dimorphic with A.

By methods which we had formerly developed it is theoretically possible to convert each of these keto esters into two racemic doisynolic acids α and β (L), one with the ethyl and carboxyl groups in the *cis* position and the other with these groups in the *trans* position. So far we have prepared five of these acids.

Acid Aα (L), derived from keto ester A, was identical with the highly active acid from the hydrogenation of the ester of acid XLVIIIα and acid Bα with the inactive one. Both of these doisynolic acids were dehydrogenated to α-bisdehydrodoisynolic acid; therefore the ethyl and the carboxyl groups must have been in the *cis* position. Acid Aβ was dehydrogenated to β-bisdehydrodoisynolic acid. It should be the racemate of doisynolic acid from estrone, and it was just half as active. Also, their mixture showed no depression of melting point. In fact, by

fractional crystallization of the cholesterol ester of Aβ we were able to obtain the (−)-antipode of the "natural" acid, which showed the same melting point, but opposite rotation.

While in the less hydrogenated doisynolic acids the activity was restricted to the *cis* forms, in the octahydrogenated A acids both diastereomers proved to be active; however, the *cis* form surpassed the *trans* form by sixty times.

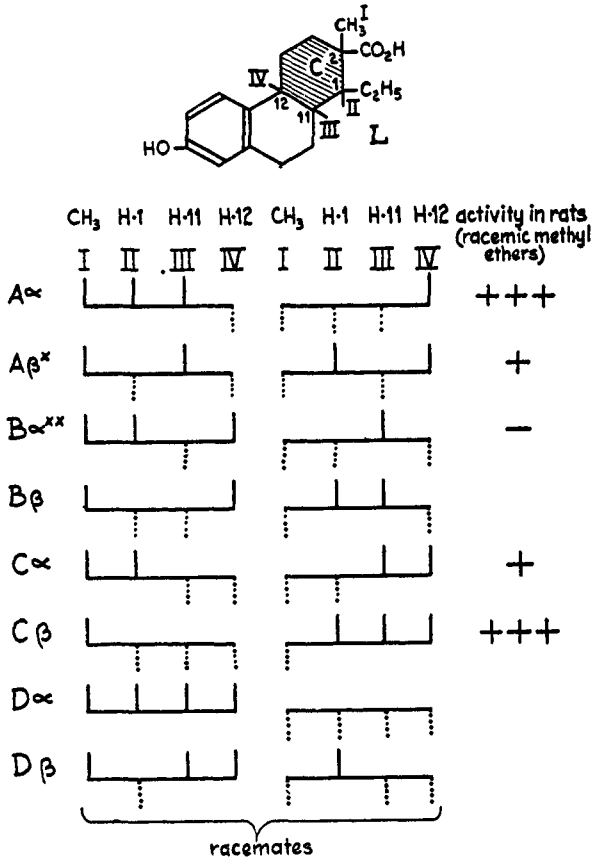


FIG. 3. Steric relationship in doisynolic acids. x = "natural" acid; xx = "lumi" acid.

The mixture of (+)-lumidoisynolic acid with L-Aα or -Cα shows a definite depression of melting point, but not with -Bα; therefore, L-Bα is probably the racemic form of lumidoisynolic acid.

Since the keto ester C (XLIX) can be transformed into the keto ester A, for instance by alkali, we assume that inversion takes place at C_{11} in the α-position to the keto group. Both acids Cα and Cβ (L) proved to be highly active but in this case the *trans* isomer Cβ, with an activity similar to that of the acid Aα, surpassed the *cis* form by about twenty times.

Figure 3 gives a short survey of the steric relationship between all possible

doisynolic acids (L). Only ring C containing all four centers of asymmetry is taken into consideration. The methyl group at C_2 and the hydrogen atoms at C_1, C_{11}, and C_{12} are numbered from I to IV. By projecting ring C to a plane vertical to its own, it is represented simply by a straight line. Substituents in the β-position (over the plane) are indicated by vertical full lines above the straight line and those in the α-position (under the plane) by dotted lines below.

Until now only those acids have proved to be active in which the substituents I and IV or 2-methyl and 12-hydrogen were in the *trans* position (as presumably in estrone). So the unknown acids Bβ and Dα and Dβ ought to be inactive or only slightly active, considering the *cis* position of the groups I and IV. From the active pairs of acids A and C those isomers (Aα and Cβ) with the hydrogens II and III in the *cis* position showed the higher activity. The same scheme may also be employed to represent the steric relationship of the isomers of estrone and a similar scheme can be used for the representation of steroids with still more asymmetric centers. An example of this is 3(α),17(β)-androstanediol

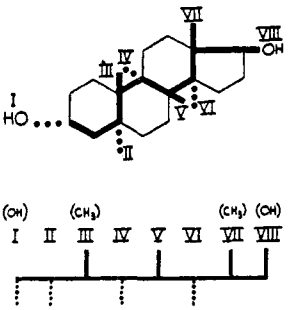


FIG. 4. 3(α),17(β)-Androstanediol (LI)

(LI), in which the centers of asymmetry are presumably as shown in figure 4.

We initially doubted the corresponding configuration of equilenin and estrone, owing to the difference in the activity of the respective monocarboxylic acids. However, this apparent inconsistency, recently also discussed by Shoppee (31), is now believed to have disappeared.

X. CONCLUSION

Our investigations starting with the monocarboxylic acid from estrone, which Doisy prepared fifteen years ago, have led to the discovery of a large class of new, highly active estrogens. They are so closely related to the natural hormones that it is interesting to speculate as to whether acids of this kind might not occur in nature; however, there is no evidence for this as yet.

It is a very curious fact that on the one hand estrogenic activity is maintained independently of whether ring D or both rings C and D of the estrogenic hormones are opened, or of whether the ring ketone or hydroxyl group is replaced in the opened forms by a carboxyl, an aldehyde, or a carbinol group; yet on the other hand, the activity is closely restricted to the presence of certain alkyl groups and especially to definite spatial configurations. So it is only natural to

look for an explanation for the established relationship between estrogenic activity and structure.

Unfortunately, we shall not be able to find such an explanation until we know more about the character of the receptor or receptors within the organism and of the processes involved in the action of the estrogens. A vast unexplored field lies ahead of us, and we can only hope that physiologists and biochemists will soon make successful advances.

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