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STUDIES ON THE COMPONENTS OF ASCLEPIADACEAE PLANTS—XXII[L]

STRUCTURES OF CYNANCHOGENIN AND SARCOSTIN

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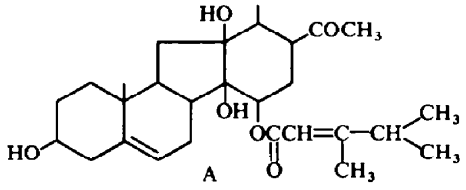
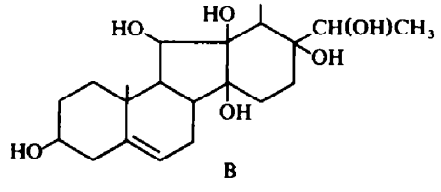
Abstract—The structures of cynanchogenin (**1**) and sarcostin (**2**) have been based on chemical and physico-chemical evidence, and the correlation of lineolon (-deacylcynanchogenin; **3**) with a conventional steroid is described. In 1959, the authors proposed the tentative structure (**A**),[2] for cynanchogenin (**1**), an aglycone from *Cynanchum caudatum* Maxim. (Asclepiadaceae).[3] In the same year, Cornforth gave the structure (**B**),[4] for sarcostin (**2**), which is also an aglycone from an Australian plant, *Sarcostemma australe*.[5] Abisch *et al.* also isolated several compounds from an African plant, *Pachycarpus lineolatus*, among which lineolon (-deacylcynanchogenin) (**3**) and sarcostin (**2**) are the main components.[6] Since then, both compounds have been isolated from many plants and have been shown to be very widely distributed steroids.*



A

(Mitsuhashi and Shimizu, 1959)



B

(Cornforth, 1959)

The C-nor-D-homopregnane skeleton of the structures **A** and **B** was assumed mainly on the basis of selenium dehydrogenation data,[7] but after our new finding on the dehydrogenation,[1b] we reinvestigated the whole problem and reached new tentative structures.† Meanwhile, Reichstein's group published a communication,[8] in which they proposed the same structures mainly on physico-chemical evidence, and this prompted us to publish a short summary of our work without conclusive evidence.[1c] Now, our success in correlating the compounds, enables us to report all the details of the structural study.[9]

Correlation of sarcostin and lineolon[1a]

SINCE penupogenin (**4**), another aglycone from *C. caudatum*, was shown to be the cinnamic ester of sarcostin,[10] biogenetically it can be assumed that both **2** and **3** have the same partial structure except for the side-chain. To clarify this point, an attempt was made to correlate cynanchogenin with sarcostin.

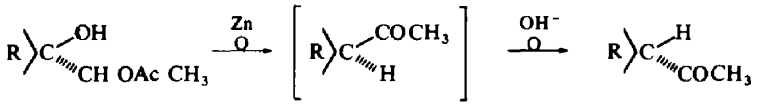
Sarcostin triacetate (**5**)[2,5,10] was submitted to the Serini reaction[11] according to the procedure by Goto and Fieser.[12]‡ The non-crystalline product was different

* To date, sarcostin has been found in more than 14 plants, and lineolon from 10 plants. Most of the plants belong to Asclepiadaceae, but some belong to completely different families.

† The contents of a thesis by Y.S., submitted to Hokkaido University in Feb., 1963, was communicated to Dr. Cornforth privately for discussion (letter on 15 Feb. 1963) and to Prof. Reichstein (letter on 11 May 1963).

‡ We are very grateful to Dr. T. Goto for the knowledge of the reaction conditions.

from lineolon diacetate (**6**) but, after alkaline hydrolysis, lineolon was obtained in a fairly good yield. This supports the assumption concerning the identity of the residues and, moreover, as the Serini reaction is known to accompany an inversion of stereochemistry,[11] the formation of lineolon only after equilibration suggests that sarcostin and lineolon have the same configuration at C-17. This relation can be expressed as shown:



5 **3** (acetate, **6**)

Skeleton[1b]

The key evidence for the C-nor-D-homoskeleton was the formation of the so-called Jacobs' hydrocarbon (**7**) by the dehydrogenation of sarcostin (**2**) and cynanchogenin (**1**),[7a] as at that time, other polyhydroxypregnanes from Asclepiadaceae plants, drevogenin[13] and condurangogenin[14] were known to afford the same hydrocarbon. Therefore, it was considered probable that a series of C-nor-D-homopregnane derivatives existed in Nature. As it was considered that normal steroids could give Jacobs' hydrocarbon (**7**) on dehydrogenation, this doubt was confirmed by finding that the C-nor-D-homo-annulation can arise very easily in pregnanes with a 12-oxygen function,[15] while this type of rearrangement is well-known in the spirostane series.[16]

3β,12β,20β-Trihydroxy-5α-pregnane (**8**), m.p. 256°, which was prepared by the sodium borohydride reduction of 3β,20β-dihydroxy-5α-pregnan-12-one (**9**),[17] was dehydrogenated with selenium. From the products, two crystalline hydrocarbons were isolated. The major compound, **10**, m.p. 148–152° proved to be a very pure sample of Jacobs' hydrocarbon (**7**).[18,*] The minor crystals, (**11**), m.p. 95–99° were unidentified. The yield of **7** was more than 10% as the pure specimen.

For comparison, a C-nor-D-homo derivative **12**[15,†] was dehydrogenated under similar conditions and the yield of Jacobs' hydrocarbon was practically the same as from **8**. The by-product is a hydrocarbon (**13**), m.p. 93° which is clearly different from **11**. This proves that the production of Jacobs' hydrocarbon does not necessarily mean the presence of a C-nor-D-homo-ring system, especially with compounds having an 12-oxygen function.‡

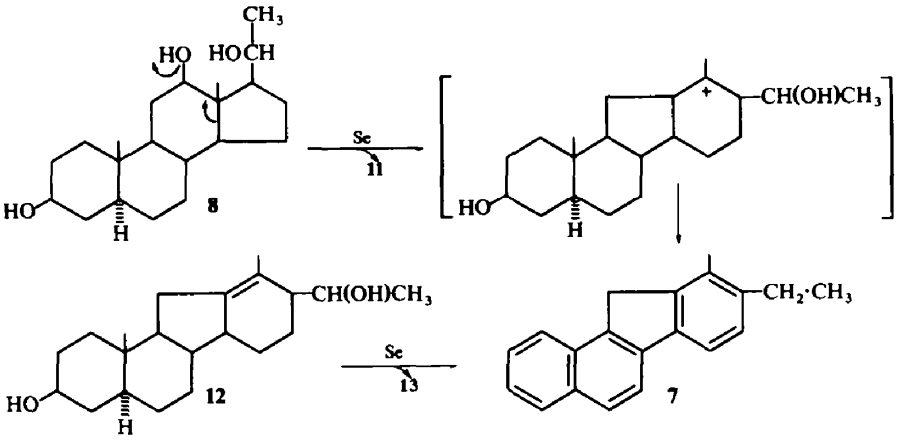
Important evidence which Cornforth proposed for the skeleton of sarcostin, is the formation of a 6-membered ring ketone (1705 cm^{-1}) by the periodate cleavage of the sarcostin side-chain.[4] This experiment was repeated with both sarcostin (**2**) and dihydrosarcostin (**14**), and the corresponding bisnor-products, **15** and **16** show absorption for a 5-membered ring ketone at ca. 1735 cm^{-1} after thorough drying of the samples§ or recrystallization from nonaqueous solvents.

* We thank Prof. T. Reichstein for his kind cooperation in the identification.

† The double bond of this compound was assigned as $\Delta^{13(17)}$, but recent investigation favors the $\Delta^{12(13)}$-structure.[19]

‡ Similar attempt was made by Reichstein's group, but **7** was not isolated.[20]

§ The same finding was shown by Reichstein's group.

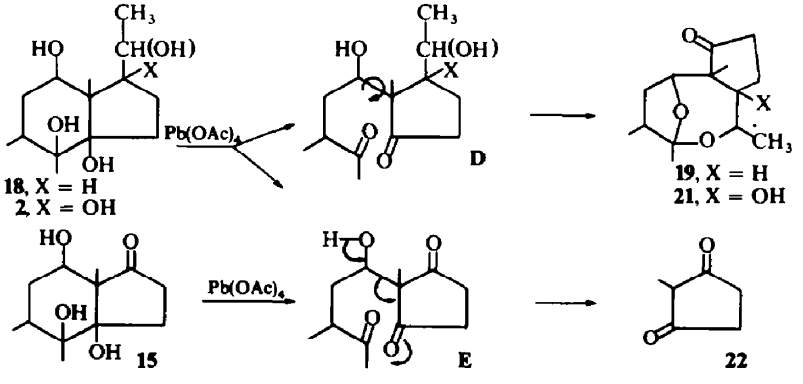


These results led us to reconsider the structures of sarcostin and lineolon in terms of a normal steroid ring system.*

Locations of oxygen functions[1c]

Lineolon possesses two secondary alcohols. One is the OH function situated at C-3, which was confirmed by formation of the Δ^4-3-ketone, **17**.[2] In addition there are a set of vicinal tertiary alcohols and a ketone at C-20.

Lead tetraacetate oxidation of the pentol in acetic acid gave a compound, **19**, $C_{21}H_{30}O_4$.[2] The IR spectrum of **19** has absorptions of a 5-membered ring ketone and an OH group, the remaining two oxygens seem to exist as a ketal (or acetal). Compound **19** was readily reduced with sodium borohydride to a keto-free ketal (**20**), $C_{21}H_{32}O_4$, m.p. 230°, which lacks a carbonyl absorption, but the presence of the ketal structure is still shown in the IR spectrum (a set of fine absorptions near 1000 cm^{-1}). This ketal formation was not observed in the oxidation of lineolon itself, whose oxygen function at C-20 is a ketone. Therefore, it is possible that one of the carbonyls derived by cleavage of the glycol forms the ketal with the 20-OH and another OH group (see the intermediate **D**). Furthermore, the formation of a 5-membered ring ketone suggests that cleavage occurred between the 5-membered

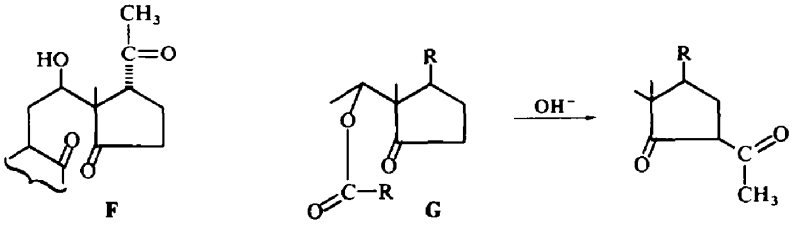


* This is also fully supported by the NMR data (*vide infra*). If the skeleton were the C-nor-D-homo-type, the 18-Me group should be split.

ring and another ring. The NMR spectrum shows two tertiary Me groups and one secondary for the 21-methyl, so there may be no skeletal rearrangement during the oxidation.

Sarcostin (**2**) gave a ketal, $C_{21}H_{30}O_5$ (**21**) and 2-methyl-cyclopentanone-1,3-dione (**22**)[4] by lead tetraacetate oxidation. The ketal formation is analogous to the case of **19** except for an additional 17-OH group. This speculation is supported by the fact that the 20-hydroxyl group participates in the ketal formation, since **2** has an OH group at C-20. But, if we assume the partial structure **C** for sarcostin, the cleavage to **E** followed by a retro-aldol reaction could give **22** without difficulty. We tried lead tetraacetate oxidation of the bisnor-derivative (**15**), where the ketal formation is not possible, and we obtained **22** in a good yield. This assumption agrees with the observation on the ketal formation; the C-8 ketone of the *seco*-diketo-compound (**D**) forms a ketal with the 12-OH group. Dihydrosarcostin behaves in a similar manner and affords **22** and a ketal, **23**.[4]

Thus, the locations of all the oxygen functions could be tentatively assigned. In the previous paper, however, it was reported that lineolon consumes about 2 moles of lead tetraacetate.[2] In the light of the new partial structure, the consumption should be 1 mole, and the oxidation product should be represented as **F**. The excess consumption may be due to some subsidiary oxidations. The lead tetraacetate oxidation product of lineolon diacetate, sarcostin triacetate, cynanchogenin and some other acetyl derivatives were found to afford enol compounds after alkaline treatment, and it was proved that an internal C-acylation is involved in the reaction.[2] In the new structure, the internal Claisen condensation on C-15 (G → H) is considered to be the only feasible interpretation.



The following reactions were an attempt to provide additional information concerning the oxygen functions.

Lineolon was submitted to Wolff-Kishner reduction to deoxolineolon (**24**), $C_{21}H_{34}O_4$, m.p. 245°. On catalytic reduction in acetic acid, **24** gave dihydrodeoxolineolon (**25**) as solvated crystals and it was oxidized with NBS, avoiding the oxidative cleavage of the glycol.* The diketo-compound (**26**), m.p. 185°, $C_{21}H_{32}O_4$ shows a composite of carbonyl absorptions at 1700 cm^{-1}, implying both secondary OH groups located on 6 (or more)-membered rings. On lead tetraacetate oxidation, **26** afforded a tetraone compound **27**, m.p. 161°, $C_{21}H_{30}O_4$, whose IR spectrum has no absorption for an OH function but one at 1737 cm^{-1} for a 5-membered ring ketone, besides absorptions at 1695 cm^{-1} and 1708 cm^{-1} for 6-membered ring ketones. Again, it was shown that the tertiary glycol is located at C-8, and C-14.

* Chromic acid oxidation results in the oxidative cleavage of the vicinal hydroxyls.[2]

Correlation of lineolon with a conventional steroid

Obviously, there is no unequivocal evidence for the skeleton and the location of all the oxygen functions, and only correlation of lineolon or sarcostin with a steroid of a known structure can provide the rigid proof.

Dehydration of lineolon or sarcostin with mineral acids resulted in a yellow pigment mixture. But when lineolon diacetate (**6**) was dehydrated with phosphorus oxychloride in pyridine at 90°, the only isolated product was an OH-free compound, **28**, $C_{25}H_{32}O_5$, m.p. 134–135°, whose UV maximum at 249 mμ suggested the $\Delta^{5\cdot8(9),14}$-triene structure. The NMR data supports this structure (τ 4·50 for 2 vinylic protons). This dehydration pattern is in agreement with other 8,14-diol systems.[21] Upon hydrolysis, this triene gave a diol, **29**, $C_{21}H_{28}O_3$, m.p. 80°/165° (double m.p.) which still maintains the same chromophore as clearly shown by the UV maximum at 249 mμ. However, reacetylation of **29** gave a different diacetate (**30**), $C_{25}H_{32}O_5$, m.p. 124–128°, λ_{max} 248 mμ. This descrepancy may be due to inversion of the 17α side-chain during base treatment to the stable 17β-orientation (*vide infra*).

Exhaustive hydrogenation of **29** gave an inseparable crystalline mixture of triols (**31**). Although this mixture is resistent to further hydrogenation, the UV spectrum shows the presence of a tetra-substituted double bond, and several attempts failed to resolve the mixture probably on account of the delicate steroisomerism at C-20. Oxidation of the mixture with chromic acid gave a trione (**32**), m.p. 203–205° as the main product. Although we considered **32** has a tetrasubstituted double bond probably at 8(14), the m.p. was close to that of 5α-pregnane-3,12,20-trione (**33**), m.p. 208°.[22] The trione (**33**) was prepared by oxidation of **8** and compared with **32**. But both have different IR spectra and show a depression in m.p. on admixture.

On the other hand, dihydroboucerin (**34**), an aglycone isolated from *Boucerosia aucheriana*, recently has been assigned the structure of 3β, 12β, 14β, 20β-tetrahydroxy-5α-pregnane by the correlation with **8** and digipurpurogenin-II.[23] On dehydration with hydrochloric acid in dioxan, **34** afforded apparently pure crystals, but as expected were shown to be a ca. 1:3 mixture of $\Delta^{8(14)}$ and Δ^{14}-isomers, **35** and **36** by NMR.[24] Chromic acid oxidation of the mixture gave crystalline products, which could be separated into two compounds using silver nitrate-impregnated silica gel TLC. The more mobile isomer (**37**), $C_{21}H_{28}O_3$, m.p. 198–200° is Δ^{14}-5α-pregnane-3,12,20-trione, as evidenced by the presence of a vinylic proton at τ 4·66. The less mobile component, **38**, m.p. 203–205°, which lacks a vinylic proton and therefore should be designated as $\Delta^{8(14)}$-5α-pregnane-3,12,20-trione, proved to be identical in all respects with the trione, **32** derived from lineolon (IR: Fig. 2).

This correlation conclusively establishes the skeleton of lineolon and the position of the oxygen functions at C-3, C-12 and C-20.

Stereochemistry

The Me signals of lineolon, sarcostin and some of their derivatives listed in the Table 1 show remarkable down-field shifts for both the 18-Me and the 19-Me. This striking influence should be attributed to the 8β-OH function, which is in the relation of 1,3-diaxial to both 18 and 19-Me groups.[25,*] The oxidation of the glycol to the

* In the case of compounds, 2 and 3, some portion of the deshielding should be attributed to the solvent effect of pyridine.

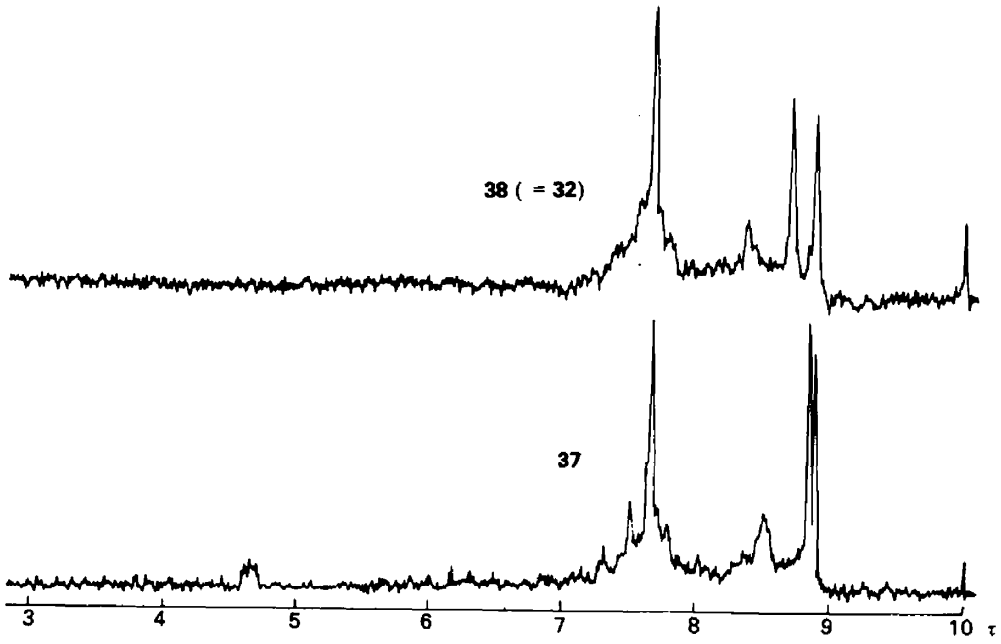


FIG. 1 NMR spectra of triketones, **37** and **38** (=**38**) in $CDCl_3$.

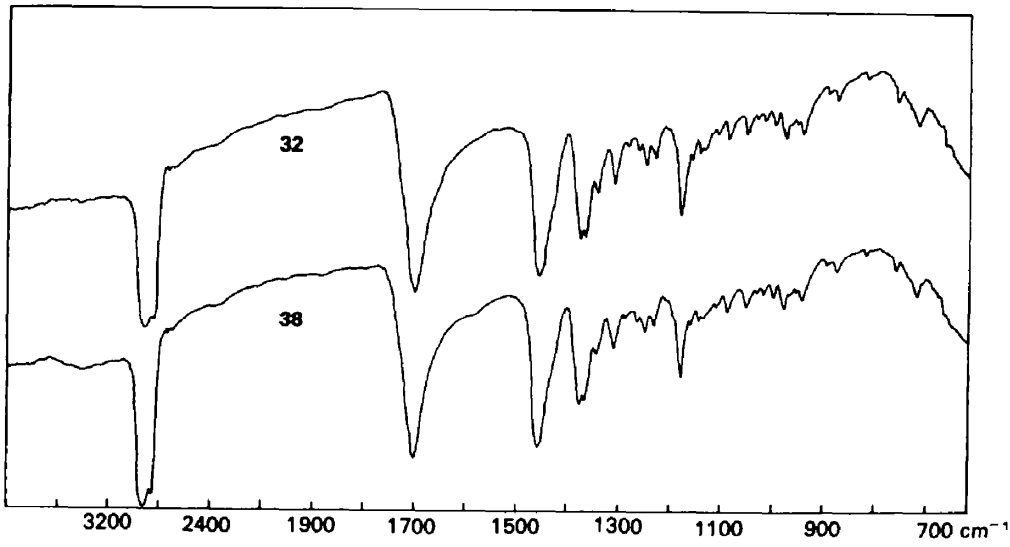


FIG. 2 IR spectra of triketones, **32** and **38** in Nujol.

Compound	Solvent	Signal (τ) 19-Me	18-Me	21-Me
Lineolon (3)	Pyridine	8·58 s	8·07 s	7·60 s
Lineolon diacetate (6)	$CDCl_3$	8·85 s	8·50 s	7·82 s
Sarcostin (2)	Pyridine	8·59 s	8·11 s	8·53 d (J = 7 c/s)
Sarcostin triacetate (5)	$CDCl_3$	8·83 s	8·58 s	8·78 d (J = 7 c/s)
Ketal (18)	$CHCl_3$	9·03 s	8·98 s	8·75 d (J = 6 c/s)
Triene (28)	$CDCl_3$	8·77 s	8·79 s	

ketal **18** relieve them from the effect. The abnormally low nature of the 18-Me signals, which can not be accounted for by the 8β-axial effect alone, might be attributed to the joined effect of oxygen functions around the Me group.

Lineolon has an unresolved triplet for one proton at τ 6·60. The origin of this signal had been uncertain, but by comparison with other spectra, it was assigned to 17α-proton. Usually, a 17-methine proton of a pregnane-20-one takes place at higher than τ 7·0. However, if the proton is β-orientated in the presence of a 14β-OH group, the signal appears near at τ 6·6 as a roughly resolved triplet. The relation is analogous to the 1,3-diaxial interaction in a cyclohexane ring. Some of the examples are listed below.* The orientations of the 14-OH group and the side-chain were also confirmed by the ORD study (*vide infra*).

TABLE 2. NMR DATA FOR 17-H SIGNALS

Compound	Solvent	Signal of 17-H (τ)
Lineolon (3)	pyridine	6·60 (unresolved triplet)
Lineolon diacetate (6)	$CDCl_3$	6·77 (unresolved triplet)
Lineolon 3-monoacetate (39)*	$CDCl_3$	6·56 (triplet)
Isodigipurpurogenin-II diacetate (= ramanone diacetate)[26]	$CDCl_3$	6·70 (unresolved triplet)
Dihydroisodigipurpurogenin-II diacetate[26]	$CDCl_3$	6·77 (unresolved triplet)
3β-Acetoxy-14β-hydroxy-5α-pregnan-20-one (17β-H)[27]	$CDCl_3$	6·80 (unresolved triplet)
3β-Acetoxy-14β-hydroxy-5α-pregnane-20-one (17α-H)[27]	$CDCl_3$	7·35

* The preparation of this compound is to be included in a future paper.

The clues for the stereochemistry of 3 and 12-OH groups were also given by NMR. The coupling constants of the hydrogen in various derivatives listed in Table 3 clearly support that both hydrogens are axial, i.e. 3β,12β-hydroxyls[28] and the coupling patterns also well-coincide with those of some compounds with known configurations at C-12.

It was already mentioned that sarcostin and lineolon have the same configuration at C-17, and that the latter is the stable isomer. When **3** was treated in an alkaline solution, an equilibrium mixture (ca. 7:3) of **3** and isolineolon (**41**),[29] m.p. 248° was formed. The ORD curve of **3** shows a negative Cotton effect, while the iso-compound (**41**) has a positive one (Fig. 3). This result is inconsistent with that of a C/D-*trans*-20-keto steroid, where the 17β-20-ketone shows a positive Cotton effect[30] and is the more stable form. But it can be understood by assuming that the 14-OH is β-oriented,

* In the case of compounds, **2** and **3**, some portion of the deshielding should be attributed to the solvent effect of pyridine.

TABLE 3. COUPLING CONSTANTS OF 3- AND 12-H

Compound	Signals (τ) 3-H (range c/s)	12-H ($J_{11\alpha\text{-H},12\text{-H}}$, $J_{11\beta\text{-H},12\text{-H}}$ c/s)
Lineolon-3-monoacetate (**39**)*	5·39 (~30)	6·22 (5, 12)
Sarcostin-3,20-diacetate (**40**)†	—	6·40 (6, 12)
Triene (**28**)	5·40 (~30)	5·07 (6, 10)
3β-Acetoxy-5α-pregnan-12β-ol*		6·54 (5, 11)
3β-Acetoxy-5α-pregnan-12α-ol*		6·16 (4, 3)
3-Oxo-14β-hydroxy-12β-tosyloxy-5β-cardenolide*		5·63 (6, 9)

* The preparation of this compound is to be included in a future paper.
† See footnote to Table 2.

i.e. C/D-fusion is *cis*, where the 17α-configuration is more stable and expected to give a negative Cotton effect.* Furthermore, 17,20-bisnor-sarcostin (**15**) has a Cotton effect similar to that of 14β-androstan-17-one.[32]

Thus the total structures of lineolon was established including the stereochemistry, and also that of sarcostin except for the configuration at C-20. Cynanchogenin is the ester of lineolon with 3,4-dimethyl-2-pentenoic acid (ikemaic acid).† The location of the ester linkage was established previously as the 12-oxygen by oxidizing **1** to the Δ^4-3-one compound (**42**), which can be further hydrolyzed to the ester-free compound (**17**).[2] At the same time, the structures of tayloron (**43**) from *Gongronema taylorii*[8] and metaplexigenin[33] from *Metaplexis japonica* were confirmed, since both compounds were connected with sarcostin.

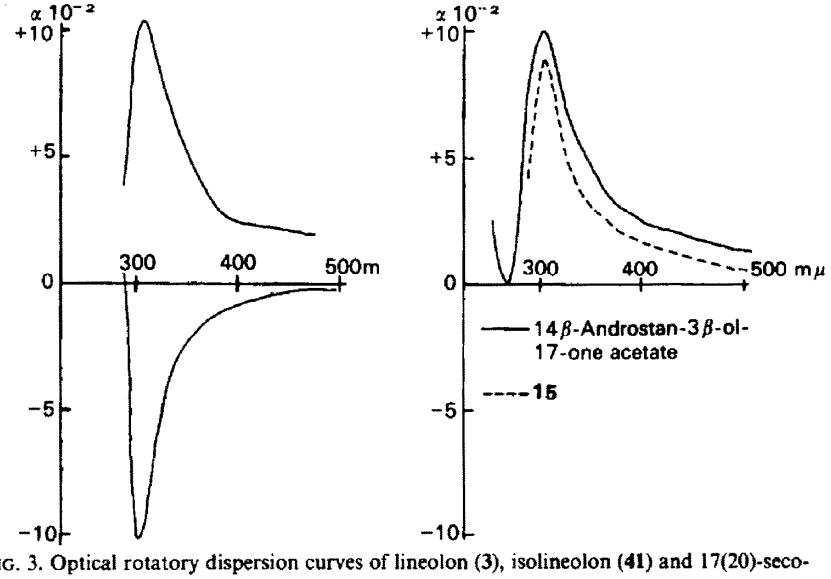
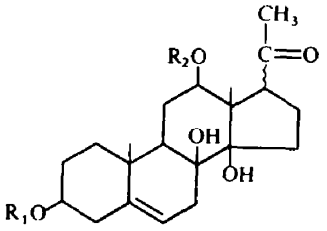


FIG. 3. Optical rotatory dispersion curves of lineolon (**3**), isolineolon (**41**) and 17(20)-seco-sarcostin (**15**) (in dioxan).

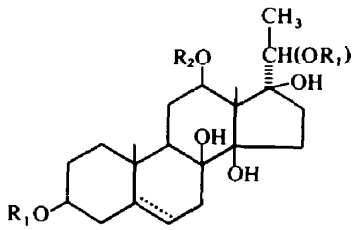
* Although the reverse equilibration-ratio has been known for a long time[31] this rationalization on the relation between ORD sign and equilibration was first made to lineolon and isolineolon by Y.S. and later extended to other steroids.[26]

† Ambiguity that cynanchogenin might be the ester of isolineolon was ruled out by the fact **1** has a negative Cotten effect. We are indebted to Mr. Shimada for the information.

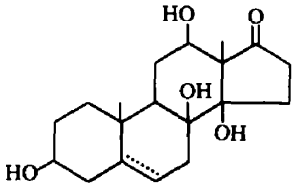


1 17α, R_1 = H, $R_2(CH_3)_2CHC=CHCO-$ (CH$_3$)

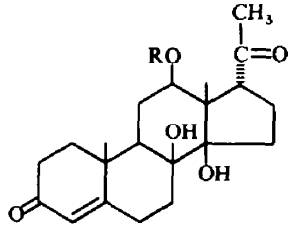
3 17α, R_1, R_2 = H
6 17α, R_1, R_2 = Ac
39 17α, R_1 = Ac, R_2 = H
41 17β, R_1, R_2 = H



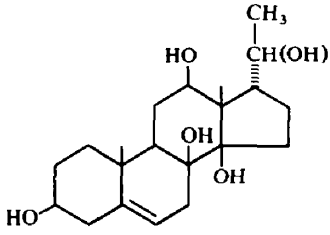
2 Δ^5-, R_1, R_2 = Ac
4 Δ^5-, R_1 = H, R_2 = ϕ—CH=CHCO—
5 Δ^5-, R_1, R_2 = Ac
14 5α-, R_1, R_2 = H
40 R_1 = Ac, R_2 = H



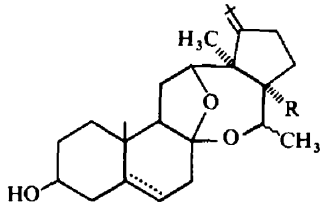
15 Δ^5-
16 5α-



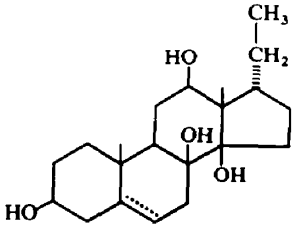
17 R = H
42 R = $(CH_3)_2CHC=CHCO-$ (CH$_3$)



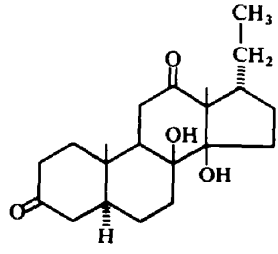
18



19 Δ^5- R = H, X = O
20 R = H, X = H, OH
21 R = OH, X = O
23 5α- R = OH, X = O



24 Δ^5-
25 5α-



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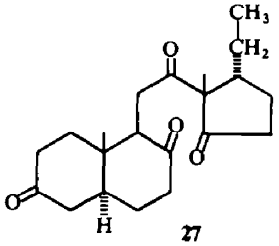

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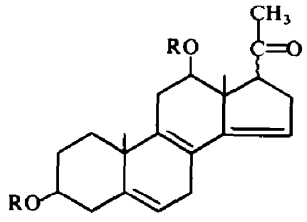
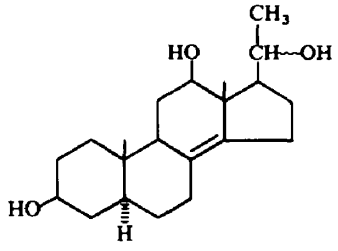
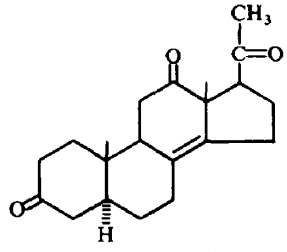


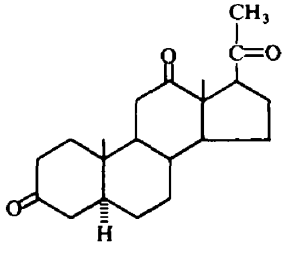
28 17α, R = Ac
29 17β, R = H
30 17β, R = Ac



31 (20α + 20β)



32 (= 38)



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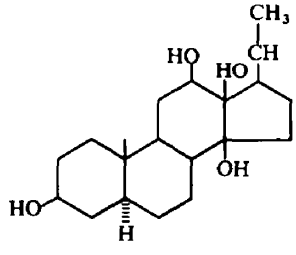

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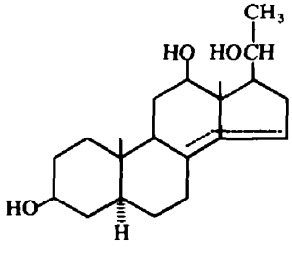
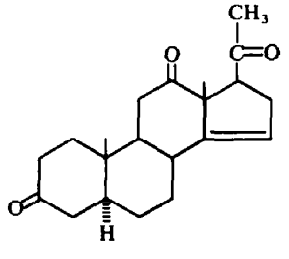


35 $\Delta^{8(14)}$
36 Δ^{14}



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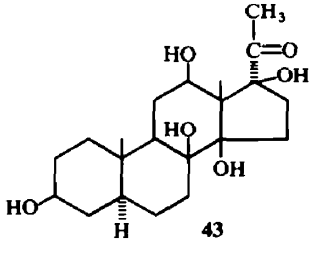

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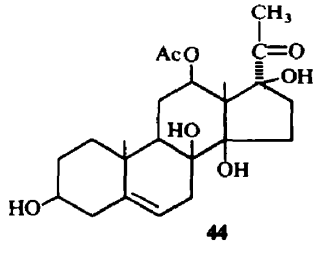

44

EXPERIMENTAL

Unless otherwise stated, UV spectra were obtained with ethanolic solns. IR spectra were taken on a Shimadzu instrument type IR. NMR spectra were obtained in Hitachi and Nihondenshi instrument (both operating at 60 Mc/s). M.ps were measured on a Kofler-block. ORD curves were taken by a JASCO instrument.

Lineolon (**3**) *from sarcostin triacetate* (**5**). Compound (**5**) was heated at reflux with Zn granules, activated by Goto and Fieser's procedure[3] in xylene under N_2 for 24 hr. The Zn was removed by decantation and evaporation of the solvent left a gummy residue. TLC examination (silica gel, 1% MeOH in $CHCl_3$) showed no spot corresponding to **6**, but a different one and other minor spots besides the starting material. The mixture was heated under reflux in 5% methanolic KOH (5 ml) for 5 hr. MeOH was removed under a reduced press and the residue was extracted continuously with ether. The ethereal extract showed the spots of **3**, **2**, **41** and some minor spots on TLC. The extract was stored in a large amount of ether to deposit prisms, which were recrystallized from acetone as prisms, m.p. 238°. Mixed m. p. with **3** showed no depression. Mobility on PPC and IR spectra are also identical.

3β,12β,20β-*Trihydroxy-5α-pregnane* (**8**). 3β,20β-Dihydroxy-5α-pregnane-12-one (4 g) was dissolved in EtOH by heating and a soln of $NaBH_4$ (500 mg) and NaOH (300 mg) in water (10 ml) was added. The mixture was left at room temp for 24 hr. Addition of water gave crystals, which were recrystallized from aqueous EtOH as needles, **8**, (2·8 g), m.p. 254–256°, identical with the sample obtained by a different route.*

Selenium dehydrogenation of 3β,12β,20β-*trihydroxy-5α-pregnane* (**8**). The triol **8** (2·4 g) was ground finely with Se metal (4·8 g) and heated under N_2 at 310° for 24 hr. After cooling, the mixture was pulverized and extracted thoroughly with ether. The ether was evaporated and the residue was extracted with n-hexane. The hexane soluble part (1·1 g) was chromatographed on alumina (40 g).

Fraction	Wt (mg)	Fraction	Wt (mg)
1, 2	— (oil)	9	28·5 (cryst)
3	95·4 (cryst)	10	25·6 (cryst)
4	190·0 (cryst)	11	52·36 (cryst)
5	65·0 (cryst)	12	50·3 (cryst)
6	86·5 (cryst)	13	26·81 (cryst)
7	83·7 (cryst)	14, 15	207·80 (cryst)
8	43·5 (cryst)	16, 17	87·00 (cryst)

Each fraction (40 ml) 1–10; n-hexane; 11–13: benzene–n-hexane 1:19; 14–17: benzene.

The fractions, 3 and 4 have a violet fluorescence and recrystallized from acetone–MeOH as plates (**11**; 70 mg), m.p. 95–99° which was not investigated further. First recrystallization of the fractions, 5–13 from acetone–MeOH gave plates, m.p. 144–152°. Further recrystallization from the same solvents gave a sample of **10** (220 mg), m.p. 148–152°, which was identified as a pure sample of Jacobs' hydrocarbon by comparison with the synthetic material.[18]

Selenium dehydrogenation of the C-*Nor*-D-*homo-compound* (**12**). The compound **12** (1·2 g) was dehydrogenated under the same condition as **8**. The n-hexane soluble part (426 mg) of the product was chromatographed on alumina (1·5 g).

Fraction	Wt (mg)	Fraction	Wt (mg)
1	— (oil)	6	50 (cryst)
2	28 (cryst)	7	35 (cryst)
3, 4	40 (cryst)	8	20 (cryst)
5	55 (cryst)	9	not weighed (yellow cryst)

Each fraction (20 ml) 1–8: n-hexane; 8–9: n-hexane–benzene (1:1).

Fraction 2 has a violet fluorescence and crystallized from acetone–MeOH to plates (9 mg), **13**, m.p. 93°. Fractions 5–8 were collected and recrystallized from acetone–MeOH as plates (110 mg), m.p. 148–152°, identical with **10** by direct comparison. Fraction 9 is a yellow crystalline solid and was not examined.

Bisnor-derivative of sarcostin (**15**). Sarcostin **2** (50 mg) was dissolved in MeOH (2 ml) containing 50 mg HIO_4. After standing at room temp, the mixture was diluted with water and extracted continuously with ether. The extract was recrystallized once from water as needles. Recrystallization from acetone-isopropyl ether gave **15** as a crystalline powder, m.p. 221–225°; ν_{max}^{Nujol} 1738 cm^{-1}, τ (in pyridine) 8·53 (s, 3H, 19-Me),

8·15 (s, 3H, 18-Me), 4·63 (unresolved tr, 1H, 5-H). The sample prepared by Cornforth using $NaIO_4$ was reported to have m.p. 223–225°.[4] Crystallization from water gave a sample, m.p. 223–224°; ν_{max}^{Nujol} 1710 cm^{-1} (1738 cm^{-1} after drying at 130°).

Bisnor-derivative of dihydrosarcostin (**16**). To a soln of **14** (100 mg) in a mixture of MeOH (5 ml) and water (1 ml) was added 1·2 equivs HIO_4 and the mixture was allowed to stand overnight. MeOH was removed under a reduced press and the resultant soln was extracted continuously with ether. The extract was recrystallized from MeOH and benzene as needles (**16**) m.p. 232–236°; ν_{max}^{Nujol} 1735 cm^{-1}. (Found: C, 67·37; H, 8·84. $C_{19}H_{30}O_5$ requires: C, 67·43; H, 8·94%).

Lead tetraacetate oxidation of pentol (**18**). The pentol **18** (400 mg) was dissolved in 90% AcOH (30 ml) and lead tetraacetate (1·5 g) was added. After standing for 4 hr at room temp, the excess oxidant was destroyed with ethylene glycol and the mixture was extracted with $CHCl_3$. The usual isolation procedure gave **19** as needles from aqueous acetone, m.p. 242°; ν_{max}^{Nujol} 3500, 1738, 1135, 1065, 880 cm^{-1}; τ (in $CHCl_3$) 9·03, 8·98 (each s, 3H, 18 and 19-Me), 8·75 (d, 3H, J = 6 c/s, 21-Me), 5·36 (m, 2H, 3 and 20-H), 4·82 (m, vinylic proton). (Found: C, 73·43; H, 8·87. $C_{21}H_{30}O_4$ requires: C, 72·80; H, 8·73%).

Sodium borohydride reduction of **19**. The ketal **19** (30 mg) was dissolved in 2 ml EtOH and $NaBH_4$ (30 mg) in water (0·5 ml) added. After addition of two drops 10% NaOH aq, the mixture was left overnight at room temp. Removal of EtOH and addition of water deposited needles, which were recrystallized from acetone–water as needles, **20**, m.p. 230° (with initial sintering at 220°); ν_{max}^{Nujol} 3300, 1135, 1102, 1065, 1010, 900 cm^{-1}. (Found: C, 72·61; H, 8·74. $C_{21}H_{32}O_4$ requires: C, 72·38; H, 9·26%).

Deoxolineolon (**24**). To a soln of Na in anhyd EtOH (4·5 ml), 100 mg of **3** (or cynanchogenin, **1**) and hydrazine hydrate (0·6 ml) were added. The mixture was heated in a sealed tube at 170° for 14 hr. After addition of water, the whole volume was concentrated under a reduced press. The needles which were separated were collected and washed with water. The filtrate was extracted continuously with ether. The extract was combined with the above crystals and recrystallized from aqueous MeOH as needles, **24**, m.p. 238–245°; IR no CO absorption. (Found: C, 71·89; H, 9·78; $C_{21}H_{34}O_4$ requires: C, 71·96; H, 9·78%).

Paper chromatography shows contamination of a minor product, which seems to be the 17-epimer.

Dehydrodihydrodeoxoxolineolon (**26**). Compound **24** (200 mg) was reduced with PtO_2 (300 mg) in a mixture of EtOH and AcOH (15 ml) for 20 hr. The catalyst was filtered off and evaporation of the filtrate gave **25** as a solvated crystalline mass, which was almost pure on PPC and used for the next reaction without purification.

To a soln of the above crystalline mass (60 mg) in a mixture of t-butanol (9 ml) and water (1 ml), N-bromosuccinimide (160 mg), and one drop of AcOH were added. The mixture was kept in the dark at 25° overnight. The soln was diluted with ether and washed with water, dil NaOH aq and water successively. Usual work up gave a product, which was crystallized from a mixture of ether and isopropyl ether as plates, **26**, m.p. 181–185°; ν_{max}^{Nujol} 1675, 1715 cm^{-1}; $\nu_{max}^{CHCl_3}$ 1705 cm^{-1}. (Found: C, 72·40; H, 9·29. $C_{21}H_{32}O_4$ requires: C, 72·38; H, 9·26%).

Seco-compound (**27**). To a soln of **26** (50 mg) in AcOH (5 ml), lead tetraacetate (100 mg) was added. The soln was allowed to stand at room temp for 4 hr. After the usual isolation, the product was crystallized from a mixture of MeOH, ether and isopropyl ether containing a small amount of water as prisms, **27**, m.p. 156–161°; ν_{max}^{Nujol} 1695, 1708 (broad), 1738 cm^{-1}. (Found: C, 71·19; H, 8·47. $C_{21}H_{30}O_4 \cdot \frac{1}{2} H_2O$ requires: C, 71·00; H, 8·79%).

Lead tetraacetate oxidation of the seco-compound (**15**). A mixture of 100 mg of **15**, 130 mg of lead tetraacetate (1·2 equivs) and AcOH (5 ml) was left at room temp for 24 hr. Water was added and the mixture concentrated under reduced press and extracted continuously with ether. The ethereal layer was extracted with dil NaOH aq. The aqueous layer was acidified with dil HCl and extracted with ether. After usual processing the ether extract gave crystals from acetone, **22**, m.p. 205–209° (with sublimation at 180°), violet coloration with $FeCl_3$. From the mother liquor a small amount of succinic acid, m.p. 186° was identified.

Dehydration of lineolon diacetate with $POCl_3$. To a soln of **6** (500 mg) in pyridine, 2 ml of $POCl_3$ was added with ice cooling. The mixture was heated on a steam bath at about 90° for 5 hr. The mixture was poured into ice water and extracted with ether. The ethereal layer was worked up as usual to afford a residue (320 mg), which was separated by TLC (silica gel HF, $CHCl_3$–EtOAc 3:1) mainly into two zones, **A** (170 mg) and **B** (100 mg), (in order of mobility) and other polar substances remained on the starting line. **B** was the unchanged starting material. **A** was recrystallized from aqueous acetone as needles **28**, m.p. 134–137°; λ_{max} 249 mμ (ε = 12,000) (with shoulders at 240 and 258 mμ; ν_{max}^{Nujol} 1735, 1708, 1635, 1250 cm^{-1}, τ (in $CDCl_3$), 8·77 (s, 3H, 19-Me), 8·79 (s, 3H, 18-Me), 7·98, 7·76, 7·90 (s, for each, 3H, acetyls and 21-Me), 5·40 (2H, vinylic protons). (Found: C, 72·93; H, 7·74. $C_{25}H_{32}O_5$ requires: C, 72·79; H, 7·82%).

Triene diol (**29**). Compound **28** (100 mg) was dissolved in 5% methanolic KOH aq (4 ml) and refluxed under N_2. After addition of water, the crystals were collected, washed and recrystallized from aqueous MeOH as needles, **29**, m.p. 165–168° (changes form at 80°); λ_{max} 249 mμ, ν_{max}^{Nujol} 3400, 1690, 1610 cm^{-1}. (Found: C, 76·63, H, 8·46. $C_{21}H_{28}O_3$ requires: C, 76·79; H, 8·59%).

Acetylation of the diol (**29**). The diol **29** (70 mg) was acetylated with pyridine (1 ml) and Ac_2O (0·5 ml). Usual work-up gave **30**, which was crystallized from acetone–water as needles, m.p. 124–128°, λ_{max} 248 mμ, ν_{max}^{Nujol} 1740, 1700 cm^{-1}. (Found: C, 72·98; H, 7·95. $C_{25}H_{32}O_5$ requires: C, 72·79; H, 7·82%).

Hydrogenation of the diol (**29**). The diol **29** (100 mg) was shaken with PtO_2 (300 mg) in AcOH (5 ml) at room temp for 24 hr. The catalyst was filtered off and the filtrate was evaporated under reduced press to dryness. The crystalline residue was recrystallized from acetone–EtOAc as prisms, which show the same mobility on TCL as **8** and melts at 205–220°; ε 210 mμ ca. 6000. Presumably it consists mainly of **35** and its 20α-isomer (**36**).

Triketone (**32**). The above mixture of **35** and **36** (60 mg) was added to a mixture of pyridine (5 ml) and CrO_3 (200 mg). The mixture was left swirling at room temp for 20 hr. EtOAc was added and the ppt was removed by filtration through Celite. The filtrate was washed with water, dil HCl, water, and dried. Evaporation of the solvent left a crystalline residue which was recrystallized from acetone–isopropyl ether as plates, **32**, m.p. 203–205°; ν_{max} 1700 cm^{-1}; τ (in $CDCl_3$) 8·89 (s, 3H, 19-Me), 8·71 (s, 3H, 18-Me), 7·64 (s, 3H, 21-Me). (Found: C, 76·86; H, 8·35. $C_{21}H_{28}O_3$ requires: C, 76·79; H, 8·59%).

5α-*Pregnane*-3,12,20-*trione* (**33**). Compound **8** (200 mg) was oxidized with CrO_3 (200 mg) in 90% AcOH (5 ml) at room temp for 10 hr. Addition of water to the mixture deposited crystals which were recrystallized from isopropyl ether–acetone as plates, **33**, m.p. 208–212° (lit., m.p. 208°); ν_{max}^{Nujol} 1700 cm^{-1}. This compound showed almost the same mobility as **32** on TLC, but differs in IR spectrum. The mixed m.p. showed a depression.

Dehydration of dihydroboucerin (**34**). Dihydroboucerin **34**[23] (100 mg) was dissolved in 4 ml dioxan 1 ml conc HCl was added. After standing at 28° for 12 hr, the mixture was diluted with water and the crystals deposited were collected. Recrystallization from acetone gave fine prisms. The NMR spectrum (see below) indicated a 1:3 mixture of **35** and **36**. Several attempts to separate the mixture were unsuccessful.

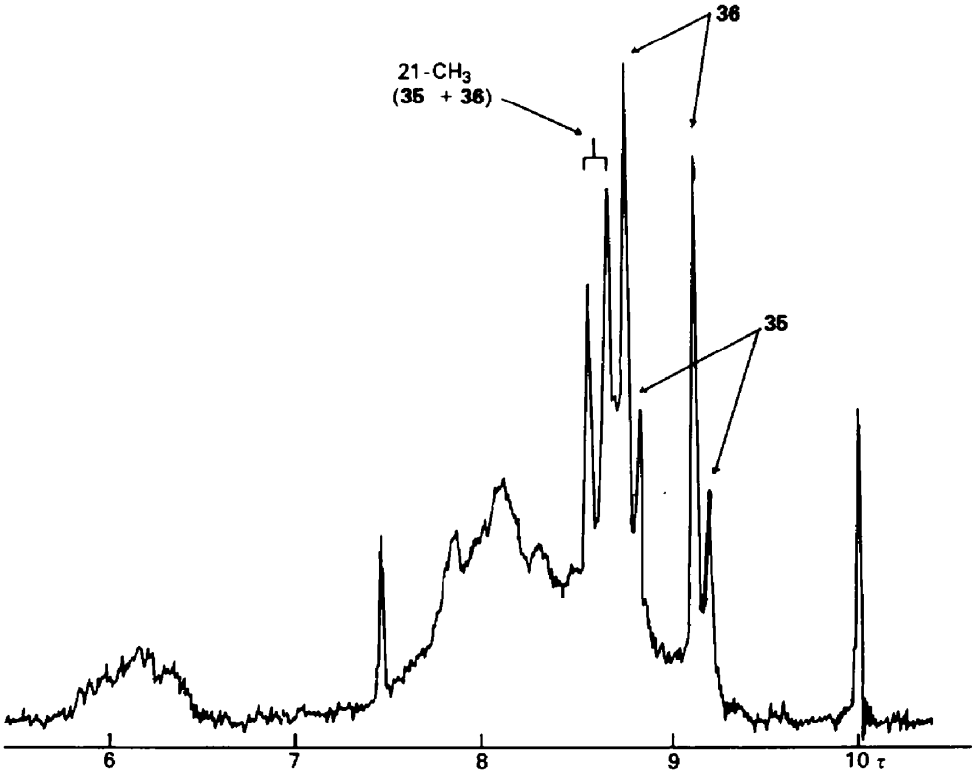

FIG. 4. NMR spectrum of the mixture of **35** and **36**.

$\Delta^{8(14)}$-5α-Pregnane-3,12,20-trione (**38**) *and* *Δ^{14}-5α-Pregnane-3,12,20-trione* (**37**). The above triol mixture (60 mg) was oxidized with CrO_3 (200 mg) in pyridine (5 ml) at room temp for 20 hr. After dilution with EtOAc, the ppt was removed by filtration through Celite. The filtrate was washed with water, dil HCl, water and dried. Evaporation gave crystals, which displayed two spots on a $AgNO_3$ impregnated silica gel TLC after multi-development using CH_2Cl_2. Preparative TLC separation by the same system afforded two products, **A** (35 mg) and **B** (14 mg) (in order of mobility).

A was recrystallized from acetone–isopropyl ether as plates m.p. 198–200°; ν_{max}^{Nujol} 1700, 805 cm^{-1}; τ (in $CDCl_3$) 8·88, 8·84 (s, for each 3H, 18 and 19-Me), 7·67 (s, 3H, 21-Me), 4·66 (m, 1H, 15-H).

B was recrystallized from CH_2Cl_2–isopropyl ether as prisms, m.p. 203–205°; ν_{max}^{Nujol} 1700 cm^{-1}, τ 8·89, 8·71 (each s, 3H, 18 and 19-Me), 7·64 (s, 3H, 21-Me) which proved to be identical with the sample **32** from lineolon.

Alkaline equilibration of lineolon (**3**). A soln of **3** (30 mg) in 5% methanolic KOH (2 ml) was warmed on a steam bath for 5 hr. The soln was diluted with water, concentrated under a reduced press, and extracted continuously with ether. The extract was submitted to partition chromatography and processed as previously reported.[29] 18 mg of **3** and 5 mg of **42**, m.p. 248–249° were obtained.

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