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STUDIES ON THE STEROIDAL COMPONENTS OF DOMESTIC PLANTS—XXXIV[1]

THE STRUCTURE OF LUVIGENIN, A NEW SAPOGENIN FROM *METANARTHECIUM LUTEO-VIRIDE* MAXIM.

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Abstract—A new steroidal sapogenin with an aromatic ring in the molecule was isolated from *Metanarthecium luteo-viride* Maxim., and proved to be 25D-19-nor-4-methyl-$\Delta^{1,3,5(10)}$-spirostatriene.

IN addition to two 11-hydroxy sapogenins, metagenin (Ia)[2] and nogiragenin (Ib)[3], *Metanarthecium luteo-viride* Maxim. contains luvigenin (II), m.p. 183–184°, $[\alpha]_D^{20}$ −34·9°, a new sapogenin having an aromatic ring in the molecule. The analytical values of luvigenin are in good agreement with the formula $C_{27}H_{38}O_2$ and its infra-red

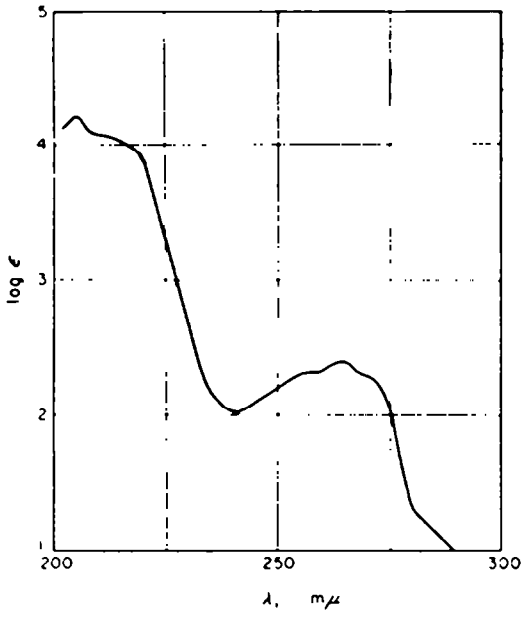
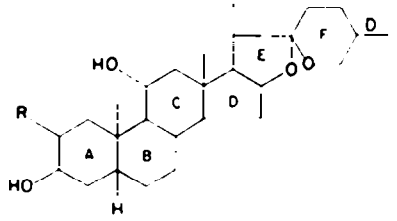


FIG. 1.

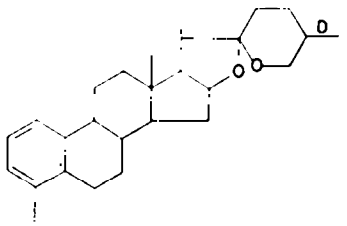
spectrum indicates not only the presence of ring E and F, but also that this sapogenin belongs to the 25D series by comparison of the intensities of the absorption bands at 923 and 900 cm^{-1}. It is interesting to note that luvigenin has only two atoms of oxygen, those present in rings E and F. This sapogenin is resistant to catalytic hydrogenation

[1] Part XXXIII: K. Sasaki, *Chem. Pharm. Bull.* In press.
[2] K. Takeda and K. Hamamoto, *Tetrahedron Letters* No. 3, 1 (1960).
[3] K. Takeda, T. Okanishi, H. Osaka, A. Shimaoka and N. Maezono, *Chem. Pharm. Bull.* In press.

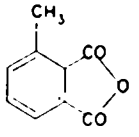
under usual conditions. The absorption maxima at 265 (ε 260) and 271 mμ (ε 200) in the ultra-violet spectrum (cf. Fig. 1) and at 785 and 740 cm^{-1} in the infra-red spectrum suggest the presence of a di- or tri-substituted benzene ring. Oxidation with 25 per cent nitric acid, although in small amount, yields toluene-2,3-dicarboxylic acid anhydride (III),[4] m.p. 113–115°, identical by mixed melting point and comparison of the infra-red spectra with an authentic specimen. Oxidation with 60 per cent nitric acid yields a mixture of benzene-1,2,3,4-tetracarboxylic acid (IVa) as its tetramethylester



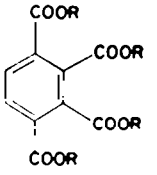
Ia R = OH
Ib R = H



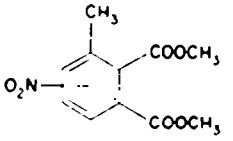
II



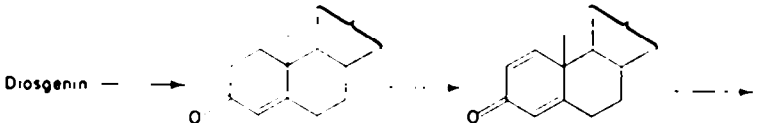
III



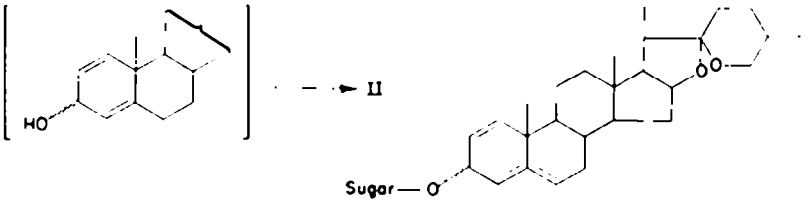
IV a R = H
IV b R = CH_3



V



VI VII



VIII IX

(VIb), m.p. 129–131°[5] (identical with the authentic specimen in all respects) together with a small amount of a nitro compound, m.p. 65–73°, which was assumed to be a mixture of x-nitrotoluene-2,3-dicarboxylic esters (V). Although these results indicate the presence of a naphthoid group in luvigenin this is not in agreement with the optical

[4] V. Jürgens, *Ber. Dtsch. Chem. Ges.* **40**, 4409 (1907).
[5] L. F. Fieser and M. A. Peters, *J. Amer. Chem. Soc.* **54**, 4347 (1932).

data. It would seem, therefore, that A ring of luvigenin is aromatic with one methyl group at C-1 or C-4 and that during oxidation aromatization of B ring takes place more readily than oxidative cleavage. Similar results have been observed by Wilds and Djerassi[6] in the oxidation of the $\Delta^{1,3,5(10)}$-cholestatriene derivative with concentrated nitric acid.

In 1958, the Scherling[7] and the Butenandt groups[8] reported independently the 1,4-dien-3-ol type rearrangement of some steroids, and in these papers they clarified that this type of rearrangement afforded the 4-methyl A-benzenoid steroid by the action of acid or magnesium silicate.

From these observations, the most probable structure for luvigenin appears to be 25D-19-nor-4-methyl-$\Delta^{1,3,5(10)}$-spirostatriene (II), and the synthesis of this compound from diosgenin has been achieved.

Diosgenin converted to diosgenone[9] was dehydrogenated to $\Delta^{1,4}$-titgogenone (VII), m.p. 200°, by the action of 2,3-dichloro-5,6-dicyanobenzoquinone.[10] This dienone was reduced with $LiAlH_4$ in tetrahydrofurane to the dienol (VIII) and treated with acetic acid without purification. The rearranged product, m.p. 183–184°, $[\alpha]_D$ −31·6°, was identical with luvigenin in all respects.

Since luvigenin has no hydroxyl group in the molecule, it may exist in the plant as a proto-type glycoside, where the aglycon takes a 1,4- or 1,5-dien-3-ol structure as represented by IX or its precursor and this proto-type sapogenin is converted to luvigenin during acid hydrolysis of the saponin mixture.

EXPERIMENTAL

All melting points are uncorrected. Unless otherwise stated, ultra-violet spectra were determined in 95% ethanol, optical rotations in chloroform, and infra-red spectra as "Nujol" mulls using a Koken Infra-red Spectrophotometer Model DS 301.

Extraction and purification of luvigenin (II)

The methanol extract of *Metanarthecium luteo-viride* Maxim. was treated with 6% ethanolic HCl on a water bath for 6 hr. After addition of water (5 l.) the ethanol was distilled off *in vacuo* and the water-insoluble product collected and saponified with methanolic KOH under reflux for 1 hr. The ether extract of the residue on treatment with cold acetone yielded crude metagenin (Ia) as a crystalline precipitate (ca. 0·4%). The filtrate was evaporated to dryness and the residue was dissolved in benzene and chromatographed.

The first eluted fraction gave luvigenin (II) as prisms, m.p. 178-180°, in 0·016% yield. Pure luvigenin, m.p. 183–184°, was obtained from methanol chloroform recrystallization: $[\alpha]_D^{28}$ −34·9° (*c* 1·130), λ_{max} 205 (*ε* 16,000), 215 (*ε* 10,200), 265 (*ε* 260) and 271 mμ (*ε* 200); ν_{max} 3080, 1585 (benzene), 981, 923 · 900, 860 (ring F), 785, 740 cm^{-1} (benzene). (Found: C, 82·45; H, 9·67. $C_{27}H_{38}O_3$ requires: C, 82·18; H, 9·71%).

Nitric acid oxidation of luvigenin

(a) 25% *Nitric acid.* A mixture of luvigenin (1 g) and 25% HNO_3 (120 ml) was heated on an oil bath for 42 hr under reflux. After cooling, the reaction mixture was extracted with ether and the aqueous layer evaporated to dryness *in vacuo*. Both the ether layer and the residue from the aqueous solution was treated with 10% Na_2CO_3, and the combined solutions acidified with HCl, and extracted with ether. From the neutral ethereal fraction, 408 mg of luvigenin was recovered.

[6] A. L. Wilds and C. Djerassi, *J. Amer. Chem. Soc.* **68**, 1712 (1946).
[7] M. J. Gentles, J. B. Moss, H. L. Herzog and E. B. Hershberg, *J. Amer. Chem. Soc.* **80**, 3702 (1958).
[8] H. Dannenberg and C. H. Doering, *Z. Physiol. Chem.* **311**, 84 (1958); **317**, 174 (1959).
[9] R. E. Marker, T. Tsukamoto and D. L. Turner, *J. Amer. Chem. Soc.* **62**, 2525 (1940).
[10] D. Burn, D. N. Kirk and V. Petrow, *Proc. Chem. Soc.* 14 (1960).

An oily acidic mixture (370 mg) was esterified with an ethereal solution of diazomethane and the ester distilled at 150-180° (bath temp) 0·4 mm. This oily ester (35 mg) was saponified and sublimed at 140–160° (bath temp) 0·1 mm. The sublimed substance was recrystallized from hexane to give needles, m.p. 113-115° (4 mg), (Found: C, 66·75; H, 3·98. $C_9H_6O_3$ requires: C, 66·67; H, 3·73%), which was identical with the authentic sample of toluene-2,3-dicarboxylic acid anhydride.

(b) 60% *Nitric acid.* A mixture of luvigenin (1·5 g) and 60% HNO_3 (45 ml) was refluxed for 24 hr. The reaction mixture was evaporated to dryness ether extracted and the residue treated with an excess of ethereal diazomethane. The resulting ester mixture was dissolved in benzene and chromatographed on alumina. The first eluted oily fraction (285 mg) was followed by a crystalline fraction. This second fraction on treatment with ether gave prisms (ca. 15 mg; IVb), m.p. 129–131°. The mother liquor was concentrated to dryness *in vacuo* and distilled under red press (b.p. 145–190° (bath temp) 0·005 mm) to give further 15 mg of the same ester, m.p. 130°. λ_{max} 208 (ε 29500) and 289 mμ (ε 1,340). (Found: C, 54·46; H, 4·66; OCH_3, 39·59. $C_{16}H_8O_4(OCH_3)_4$ requires: C, 54·19; H, 4·55; OCH_3, 40·00%). This was identical with synthetic tetramethyl mellophanate, m.p. 129–131°, by mixed m.p. and infra-red spectrum.

The first eluted oily fraction was dissolved in pet ether benzene (1:1), rechromatographed, and was separated into two fractions: the pet ether-benzene fraction and the benzene fraction.

The first fraction distilled at 140-145° (bath temp 0·004 mm), and the distillate (90 mg) purified by charcoal and crystallized from a pet ether and ether mixture afforded prisms, m.p. 65–73° (Monoscope). Further recrystallization of this substance from the same solvent did not show any change in m.p. Infra-red spectrum showed the following bands: ν_{max} 1738 (ester), 1608 (benzene) and 1538 and 1360 cm^{-1} (nitro group). From the presence of the absorption bands corresponding to the nitro group and the analytical values of this substance, it was assumed that this substance is a mixture of the position isomer of the nitro group in V. (Found: C, 51·84; H, 4·39; N, 5·96. $C_{11}H_{11}O_4N$ requires: C, 52·17; H, 4·38; N, 5·53%). Further a small amount of V and a crystalline substance, m.p. 73–76°, were obtained from the mother liquor. The latter was insufficient for further examination. Another crystalline substance (2 mg), m.p. 90–98°, was isolated from the second benzene eluate but this so far has not been examined.

Luvigenin from diosgenin

$\Delta^{1,4}$-*Tigogenone* (VII). Diosgenone (8 g) obtained from diosgenin by the Oppenauer oxidation,[9] was dehydrogenated with 2,3-dichloro-5,6-dicyanobenzoquinone (6 g) in absolute benzene under reflux for 33 hr until paper partition chromatography indicated the disappearance of diosgenone. After filtration, to the benzene solution (concentrated to half volume) ether (300 ml) and 2% KOH solution (300 ml) was added and the ether layer washed and dried. After removal of the solvent, the residue was purified by chromatography, followed by recrystallization from methanol; VII, m.p. 198–200°, yield 5·96 g (75%). $[\alpha]_D^{30}$ 78·7° (c 1·045): λ_{max} 245 mμ (ε 15,500). (Found: C, 79·13; H, 9·34. $C_{27}H_{38}O_3$ requires: C, 78·98; H, 9·33%).

1,4-*Dienol* (VIII) *and its rearrangement.* To a solution of VII (6 g) in a mixture of absolute ether (200 ml) and absolute tetrahydrofurane (40 ml) $LiAlH_4$ (3 g) in the same solvent was added at 0–5°. The mixture was heated under reflux for 1 hr, then ethanol and water were added and the organic layer yielded crude dienol (6 g, VIII). The mixture of this crude VIII (6 g), acetic acid (500 ml) and water (50 ml) was refluxed for 5 min and allowed to stand overnight. The crystalline precipitate was collected and recrystallized from methanol to give 1·7 g of the rearrangement product, m.p. 183–184°. Further addition of water (1·5 l.) to the mother liquor afforded another 363 mg of the same product. $[\alpha]_D^{27}$ 31·6° (c 1·065). (Found: C, 82·07; H, 9·87. $C_{27}H_{38}O_3$ requires: C, 82·18; H, 9·71%). This was identical with luvigenin by mixed m.p. and infra-red spectra. The above-mentioned mother liquor was evaporated to dryness and the residue was purified by chromatography. Elution with pet ether benzene (10:1) afforded 163 mg of $\Delta^{3,5}$-25D-spirostadiene, m.p. 163-165°, $[\alpha]_D^{29}$ −178·8° c 1·025), λ_{max} 228 mμ (ε 22,800). (Found: C, 81·71; H, 10·26. $C_{27}H_{40}O_2$ requires: C, 81·76; H, 10·17%),[11] while with pet ether–benzene (1:1) yielded 290 mg of diosgenone.

[11] H. Nawa, *Proc. Japan Acad.* 33, 570 (1957).