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STEROID SAPOGENINS—XIII.

THE CONSTITUENTS OF *BALANITES AEGYPTIACA*

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Abstract—The leaves and fruit kernels of *Balanites aegyptiaca* L. were found to contain six diosgenin glucosides including di-, tri- and tetraglucosides. Hydrolysis of the saponins gave 25D-spirosta-3,5-diene and 3β-chloro-25D-spirost-5-ene as artefacts accompanying diosgenin. The unsaponifiable fraction of the leaf fat contains two C_{29} sterols, one of which has an 8:14 double bond as suggested by mass spectrometry.

Balanites aegyptiaca L. (Zygophyllaceae) is a wild tree common in the Egyptian deserts, in several Middle East and African countries and in India. Kon and Weller[1] isolated the tetraglucoside of a C_{27} steroid sapogenin (named "nitogenin") from the fruit kernels and, in a later study, Marker *et al.*[2] showed that the genin was actually diosgenin, a fact which seems to have been overlooked by El-Mangouri *et al.*[3] who reported more recently the isolation of "nitogenin" from the same source. In the present report we give the results of an investigation of sapogenins isolated from various parts of a locally grown plant.

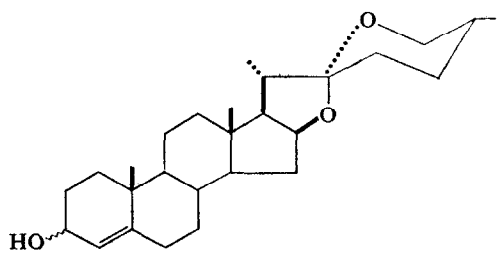
The saponins in the leaves were isolated[4] from the defatted aqueous-alcoholic extract by taking up with butanol, followed by several purification stages including the formation of the cholesterol complex. The saponin mixture was found, by TLC, to comprise six products all of which were shown by acid hydrolysis to be conjugates of diosgenin with glucose. By the use of preparative chromatoplates it was possible to isolate the three less polar saponins in pure state. Estimation of the glucose content[5] in each by colorimetric measurement[6] of the free sugar in the acid hydrolysate revealed that they contained 2, 3 and 4 glucose units respectively; the remaining saponins are undoubtedly higher glucosides. In the same manner, the fruit kernels were shown to have the same saponin content as the leaves.

Following acid hydrolysis of the saponin matter of the leaves, it was found that diosgenin was accompanied by a binary mixture of nonpolar spirostanes. They were resolved by TLC into the following: (1) A nonhydroxylic compound, m.p. 165–167°, which contained a conjugated heteroannular diene system and which was shown, by physical evidence and direct comparison, to be 25D-spirosta-3,5-diene. It is clearly not a natural constituent of the plant but an artefact resulting by acid-induced dehydration of diosgenin during hydrolysis.[7] A study of the mass spectrum of this compound revealed all the general modes of fragmentation

[1] A. R. KON and W. T. WELLER, *J. Chem. Soc.* 800 (1939).
[2] R. E. MARKER, R. B. WAGNER, P. J. GOLDSMITH, P. R. ULSHAFER and C. H. RUOF, *J. Am. Chem. Soc.* **65**, 1248 (1943).
[3] Y. M. DESSOUKY and H. A. EL-MANGOURI, *Reps Pharm. Soc. Egypt.* **35**, 12 (1953).
[4] T. KAWASAKI and I. NISHIOKA, *Chem. Pharm. Bull. (Japan)* **12**, 1311 (1964).
[5] T. KAWASAKI and T. YAMAUCHI, *Chem. Pharm. Bull. (Japan)* **10**, 698 (1962).
[6] M. DUBOIS and J. K. HAMILTON, *Nature* **168**, 167 (1951).
[7] M. S. BEDOUR, D. EL-MUNAJJED, M. B. E. FAYEZ and A. N. GIRGIS, *J. Pharm. Sci.* **53**, 1276 (1964).

expected[8] to originate from the spirostane side-chain but none which could specifically be attributed to the diene system. (2) A chlorine-containing compound, m.p. 209–212°, the nature of which (as 3β-chloro-25D-spirost-5-ene) was revealed by direct comparison with a synthetic preparation obtained from diosgenin by treatment with thionyl chloride. The mass spectrum of this compound exhibited all the usual modes of fragmentation of a typical spirostane derivative[8] and most of the fragment ions appeared in chlorine-containing and dehydrochlorinated variants.

It was thought that this chloro compound might arise during saponin hydrolysis as another artefact by the action of hydrochloric acid on the accompanying 3,5-diene. This, however, was disproved experimentally by treating the diene with hydrogen chloride in ether when no trace of the chloro derivative was obtained but, instead, a mixture of two hydroxylic 25D-spirostene compounds resulted. The mixture was readily transformed into a single product identified as 25D-spirost-4-en-3-one by chromic acid oxidation under mild conditions which would not similarly affect diosgenin. Thus, the original binary mixture of hydroxylic compounds comprised 25D-spirost-4-enols, namely allodiosgenin (I) and 3-epiallodiosgenin (II), which differ only in the configuration of the C-3 hydroxyl group. Their formation may be the outcome of a 1,4-addition of water (traces in the reaction mixture) either directly or



I (C—3α), II (C—3β)

via a primary addition of hydrogen chloride followed by hydrolysis. This type of reaction finds analogy[9] in the preparation of allocholesterol and 3-epiallocholesterol from cholesta-3,5-diene by a similar treatment. It is thus seen that 25D-spirosta-3,5-diene and 3β-chloro-25D-sprisot-5-ene are two artefacts accompanying diosgenin and result from it during hydrolysis of the natural saponins with hydrochloric acid. The formation of the chloro compound directly from diosgenin has been proven experimentally by Yamauchi[10] by treatment with this acid under the usual hydrolysis conditions.

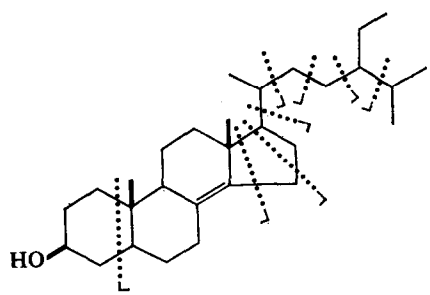
In isolation experiments, it was found that diosgenin occurs to the extent of 0·30 per cent in the fruits and only 0·08 per cent in the flowering stalks of the plant.

The unsaponifiable fraction of the leaf fat afforded a sterol material (m.p. 157–160°) which was seemingly uniform as judged by TLC on silica gel plates and by inspection of the acetyl derivative (m.p. 137–139°) on silver nitrate-impregnated silica gel[11] plates. The existence of two components in the isolated material was, however, revealed from a study of the mass spectrum of the acetyl derivative which showed two molecular ion peaks (*m/e* 456 and 454) corresponding to the alcohols $C_{29}H_{50}$ and $C_{29}H_{48}O$, containing one and two ethylenic linkages.

[8] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Monatsh.* **93**, 1033 (1962).
[9] J. C. ECK and E. W. HALLINGSWORTH, *J. Am. Chem. Soc.* **63**, 2320 (1941).
[10] T. YAMAUCHI, *Chem. Pharm. Bull. (Japan)* **7**, 343 (1959).
[11] J. W. COPIUS-PEEREBOOM, *Z. Anal. Chem.* **205**, 325 (1964).

Apart from the common expulsions of methyl radical (probably from C-18[12]) and acetic acid, the spectra showed the following fragmentations which all arise from the deacetoxylated (M^+-60) ions (*m/e* 396 and 394) of both products. The losses of isopropyl group (substantiated by metastable ions) giving peaks at *m/e* 353 and 351 and those of C_6H_{13} (*m/e* 311 and 309), C_7H_{15} (*m/e* 297 and 295), C_8H_{17} (*m/e* 283 and 281, supported by metastable ions) as well as the entire side-chain (*m/e* 255 and 253, supported by metastable ions) prove that both compounds carry a saturated $C_{10}H_{21}$ side-chain and that both sterols have a stigmastane skeleton. The loss of the side-chain along with 42 mass units[13] was also observed giving appreciable peaks at *m/e* 213 (supported by a metastable ion) and 211. The presence of the fragment ions *m/e* 201 and 159, resulting by loss of 54 mass units from products *m/e* 255 and 213 of the singly unsaturated sterol (with counterparts from the other one) through collapse of ring A in a retro-Diels–Alder reaction,[13] proves that neither sterol contains a double bond in the 5:6-position. The presence of an appreciable ion at *m/e* 228, formed from *m/e* 396 by the total loss of the side chain and 27 mass units (supported by a metastable ion), may be an evidence for the location of the single double bond in the $C_{29}H_{50}O$ sterol at C-8:C-14. This assignment would be favourable for the cleavage of the C-13/C-17 and C-15/C-16 bonds to give the ion *m/e* 228 and has no counterpart for the doubly unsaturated sterol.

The above data, therefore, indicate that one component ($C_{29}H_{50}O$) of the natural sterol mixture in *B. aegyptiaca* L. leaves may be stigmast-8(14)-en-3β-ol (III) or a close isomer and that the other ($C_{29}H_{48}O$) is a stigmastadienol of undecided constitution.



(III)

EXPERIMENTAL

The m.ps. are uncorrected. The optical rotations were measured in $CHCl_3$, the u.v. spectra were determined in EtOH and the i.r. spectra in CS_2. The mass spectra were determined (unless otherwise stated) with a CH_4 instrument at 70° using an electron energy of 70 eV. For TLC, silica gel G was used (unless otherwise stated) and development was made with the following solvent systems: I, $CHCl_3$–MeOH–water (13:7:5, lower phase); II, toluene–$CHCl_3$–*iso*propanol (10:1:1); III, *n*-hexane–EtOAc (20:1); IV, *iso*propanol–toluene-EtOAc–water (20:4:10:5); V, *n*-hexane–EtOAc (2:1); VI, *n*-hexane–EtOAc (5:1); VII, $CHCl_3$–light petroleum–AcOH (5:15:0·1). The chromatograms were sprayed with *p*-anisaldehyde–AcOH–H_2SO_4 (100:1:2) for saponins and sterols, with chlorosulphonic acid–AcOH (1:3) for sapogenins, and with dipheny amine–aniline (2% acetone solution of each)–phosphoric acid (9:9:2) for sugars.

Isolation of the Saponins

The concentrated EtOH extract of the leaves was defatted with benzene and, after addition of NaCl and little HCl, the saponins were extracted with wet *n*-BuOH. The dry BuOH extract was reextracted with MeOH,

[12] N. S. WULFSON, V. I. ZARETSKII, V. G. ZAIKIN, G. M. SEGAL, I. V. TORGOV and T. P. FRADKINA, *Tetrahedron Letters* 3015 (1964).

[13] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAM, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. 2, 21, Holden-Day, San Francisco (1964).

charcoaled and, after evaporation and taking up with $CHCl_3$–90% EtOH (1:2), an EtOH solution of cholesterol was added and the mixture boiled for 20 min. After standing, the complex precipitate was washed with EtOH and ether, then heated in pyridine at 100° for 1 hr. The solid formed upon addition of excess ether was extracted (Soxhlet) with $CHCl_3$–MeOH (1:1) and the extract (charcoaled) afforded a mixture (1·45 per cent of the dry plant weight) which was shown (TLC, system I) to comprise six saponins: R_f 0·55, 0·45, 0·35, 0·30, 0·20, and 0·10.

The Sugar Content

The saponin mixture, hydrolysed with 2 N H_2SO_4 in aq. EtOH, gave diosgenin (R_f 0·39; TLC, system II) from which the acetate (TLC, silver nitrate-impregnated alumina, system III, R_f 0·30) was prepared. The neutralized aqueous hydrolysate ($BaCO_3$) showed only D-glucose (R_f 0·63; TLC system IV).

The saponin mixture (above) was fractionated by TLC (system I, using iodine vapour to localize the zones) which enabled the isolation of only those with R_f 0·55, 0·45 and 0·35 as amorphous deliquescent pure solids. A known weight of each saponin was hydrolysed (2 N H_2SO_4 in aq. EtOH, 2 hr reflux) and the weight of resulting diosgenin recorded. The content of glucose in the aqueous hydrolysate was also determined colorimetrically,[6] after addition of 5% aq. phenol solution and conc. H_2SO_4, at 490 nm. The three saponins were thus found to contain 43, 59 and 69 per cent glucose which correspond to 2, 3 and 4 glucose units to each diosgenin molecule.

The saponins of the fruit kernels were studied in exactly the same manner as the leaves and were found to be qualitatively identical.

Fractionation of the Sapogenins

In another experiment, the *n*-BuOH saponin extract (above) from the leaves was concentrated then hydrolysed with 4 N HCl and the $CHCl_3$ extract of the aglycone matter purified by a brief reflux with MeOH-alkali. The resulting sapogenin mixture (1·75 per cent of dry plant weight) was fractionated on an alumina column to give (by elution with *n*-hexane) a mixture (0·075 per cent of dry plant weight) which was shown by TLC (system III) to comprise two products (R_f 0·72 and 0·61). It was followed by diosgenin (eluted with 1% EtOH in *n*-hexane, 0·49 per cent of dry plant weight), m.p. and mixed m.p. 210–212°, $[\alpha]_D$ −126° (reported[2] m.p. 206°, $[\alpha]_D$ −120°) from which the acetate (m.p. and mixed m.p. 198–200°, $[\alpha]_D$ −121°; reported[2] m.p. 200°, $[\alpha]_D$ −115°; identical i.r. spectra) was prepared.

The mixture eluted with *n*-hexane was resolved by TLC (same conditions, using iodine vapour to localize the zones) into two components. The first (R_f 0·72) was obtained as needles ($CHCl_3$–MeOH), m.p. 165–167° undepressed by 25D-spirosta-3,5-diene (reported[14] m.p. 164°), which gave orange tetranitromethane reaction (u.v. spectrum: triplet at 228, 235 and 245 (shoulder) nm, ϵ = 28,000, 285,000, and 18,700; i.r. spectrum: bands at 868, 900, 920 and 985 cm^{-1}, with the second stronger than the third, 25D sapogenin side-chain[15]). The mass spectrum contained peaks at *m/e* 396 (M^+), 381 (M^+-CH_3), 337, 327 and 324 (losses from ring F), 282, 267 and 253 (losses from rings E and F), 213 (loss of ring D and side-chain) and at 139 (100 per cent) and 115 (ions comprising ring F).[13]

The second product (R_f 0·61) afforded needles ($CHCl_3$–MeOH), m.p. 209–212° (undepressed by synthetic preparation, see later; reported[10] for 3β-chloro-25D-spirost-5-ene, m.p. 213°), which gave yellow tetranitromethane reaction. The mass spectrum contained peaks at *m/e* 434 (M^+), 373, 363 and 360 (losses from ring F), 318, 303 and 289 (losses from rings E and F), 249 (loss of ring D and side-chain) which were all accompanied by satellite peaks resulting from loss of HCl. The spectrum also contained peaks at *m/e* 139 and 115 (100 per cent intensity, ions comprising ring F).[13] Treatment of this compound with 2% EtOH–NaOH under reflux for 3 hr gave a hydroxylic product with the same R_f as diosgenin (TLC, system II, R_f 0·39). A synthetic sample was prepared by treatment of diosgenin (0·3 g) in benzene (dry, 10 ml) with $SOCl_2$ in benzene (60 per cent, 8 ml) at room temperature for 3 hr. The residue from the reaction mixture was chromatographed on alumina to give 3β-chloro-25D-spirost-5-ene (0·21 g), m.p. 208–211°, $[\alpha]_D$ −109° (reported[10] $[\alpha]_D$ −101°), by elution with *n*-hexane.

Action of Hydrogen Chloride on 25D-Spirosta-3,5-diene

A stream of dry HCl gas was passed through a solution of 25D-spirosta-3,5-diene (0·5 g) in ether (50 ml) at 3–6° for 3 hr. The residue from the reaction mixture was chromatographed on an alumina column to give some unchanged material by elution with *n*-hexane followed by a mixture (0·15 g, R_f 0·67 and 0·78, diosgenin R_f 0·67, system V) by elution with benzene. The material gave positive digitonin and negative halogen tests; i.r. bands at 865, 900, 920 and 985 cm^{-1} with the second stronger than the third (25D sapogenin side-chain) and near 3550 cm^{-1} (OH).

The above mixture (90 mg) in acetic acid (5 ml) was treated with chromic anhydride in acetic acid (8 N,

[14] M. E. WALL and S. SEROTA, *J. Am. Chem. Soc.* **78**, 1747 (1956).
[15] C. R. EDDY, M. E. WALL and M. K. SCOTT, *Anal. Chem.* **25**, 266 (1953).

3 ml) for 5 min and the product obtained was chromatographed on alumina. This gave, by elution with *n*-hexane, colourless needles from acetone–MeOH (30 mg), m.p. 178–181°, $[\alpha]_D - 1°$, identified as 25D-spirost-4-en-3-one (reported[16] m.p. 186°, $[\alpha]_D \pm 0°$); u.v.: peak at 240 nm ($\epsilon = 14{,}500$), i.r.: band near 1680 cm^{-1} ($\alpha\beta$-unsaturated C=O).

Constituents of the Fruits and Flowering Stalks

These organs were processed in essentially the same manner as the leaves and were found to contain 0·30 per cent and 0·08 per cent diosgenin (dry weight basis) respectively.

The Sterols of the Leaf Fat

The fat-containing benzene extract (resulting during the processing of the leaves, above) was saponified and the unsaponifiable fraction (0·55 per cent of dry plant weight) chromatographed on alumina. Elution with benzene gave needles (0·023 per cent of dry plant weight), m.p. 157–160°, of a sterol material (specific colour tests), R_f 0·44 (system VI), from which the acetate (m.p. 137–139°, R_f 0·35 silver nitrate-impregnated plates, system VII) was prepared. The mass spectrum (cf. Theoretical) was measured on an AEI MS9 instrument.

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[16] R. E. MARKER, T. TSUKAMOTO and D. L. TURNER, *J. Am. Chem. Soc.* **62**, 2525 (1940).