243. *Saponins and Sapogenins. Part IV.* Agapanthagenin (22a-Spirostan-2α : 3β : 5α-triol), a New Sapogenin from* Agapanthus *Species.*

By (Mrs.) T. Stephen.

From several *Agapanthus* species a small quantity of yuccagenin (22a-spirost-5-en-2α : 3β-diol) has been isolated whilst the main constituent is a new steroidal sapogenin, $C_{27}H_{44}O_5$, for which the name agapanthagenin is proposed. The compound is considered to be 22a-spirostan-2α : 3β : 5α-triol.

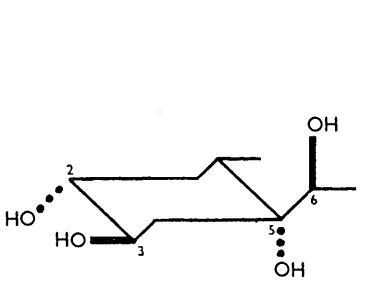
Agapanthagenin has three hydroxyl groups, one of which is considered to be tertiary since only a diacetate is formed, and this contains a free hydroxyl group. The infrared absorption spectrum of the diacetate shows agapanthagenin to be a true steroidal sapogenin with an *iso*-configuration of ring F. The acetate band at 1244 cm.$^{-1}$ closely resembles that recorded [1] for gitogenin diacetate (5α : 22a-spirostan-2α : 3β-diol diacetate) and, in addition to indicating the two hydroxyl groups to be at $C_{(2)}$ and $C_{(3)}$, the simplicity of this band suggests a *trans*-fusion of rings A and B. Agapanthagenin and its diacetate are saturated (tetranitromethane test and the ultraviolet absorption spectrum [2]) and in boiling 2N-ethanolic hydrochloric acid develop a strong green-yellow fluorescence indicative of

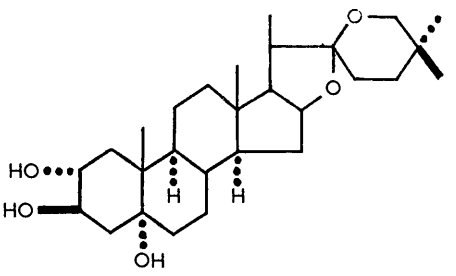
* Part III, *Chem. and Ind.*, 1954, 727.

[1] Eddy, Wall, and Scott, *Analyt. Chem.*, 1953, **25**, 266.
[2] Bladon, Henbest, and Wood, *J.*, 1952, 2737.

dehydration [3] and yield a crystalline compound $C_{54}H_{86}O_9$. Boiling 1N-ethanolic hydrochloric acid hydrolyses the diacetate to agapanthagenin.

Analyses of the compound $C_{54}H_{86}O_9$ agree with its formulation as an equimolar complex of agapanthagenin and its dehydration product. The formation of stable molecular complexes in the steroid field is well established.[4] Chromatographic analysis of the complex failed to separate it into its components, but acetylation and fractional crystallis-





ation gave agapanthagenin and yuccagenin diacetates. The complex and yuccagenin diacetate derived from agapanthagenin are both identical with the corresponding compouunds isolated from the plant, and the infrared absorption spectrum of yuccagenin diacetate agrees with that recorded by Eddy, Wall, and Scott.[1] Chromatographic analysis of the acetylated complex gave (*a*) an isomer of yuccagenin diacetate, indicting that a shift of the double bond may have taken place, and (*b*) agapanthagenin diacetate. Agapanthagenin diacetate may be dehydrated directly to yuccagenin diacetate thus indicating that the free hydroxyl group takes part in the dehydration; being tertiary in character this hydroxyl group must be situated at $C_{(5)}$. The specific spatial configuration of this group is derived by comparing the dehydration of agapanthagenin with the behaviour of the two isomeric ergostadiene-3 : 5 : 6-triols.[5] Triol I and hydroxyergostanedione, in which rings A and B have a *trans*-decalin configuration, are readily dehydrated with hydrogen chloride; whereas triol II which is a *cis*-decalin derivative does not lose water even on prolonged treatment of its diacetate with phosphoric oxide. Petrow, Rosenheim, and Starling [6] have likewise shown that 3β : 6β-diacetoxycholestan-5α-ol readily loses water, yielding cholest-4-ene-3β : 6β-diol diacetate. That the triol II could not be dehydrated can only be attributed to the specific spatial configuration of the tertiary $C_{(5)}$-hydroxyl group in *cis*-decalin derivatives. The assignment of 22a-spirostan-2α : 3β : 5α-triol to agapanthagenin receives further support from the ready dehydration which is to be expected if rings A and B are *trans*-fused. The $C_{(5)}$-hydroxyl group is then polar and consequently can be eliminated with the coplanar $C_{(6)}$-hydrogen.

Experimental

Rotations were determined in chloroform at 25°. M. p.s were determined on a block and are corrected. The alumina used for chromatography was washed and activated at 180°.

Isolation of Sapogenins.—Minced, fresh *Agapanthus* rhizomes (1000 g.) and 2N-hydrochloric acid (1000 c.c.) were boiled for 4 hr. The hydrolysed saponins and the residual plant material were filtered off, washed free from acid, and dried at 100° for 72 hr. The dried cake was ground and extracted continuously for 6 hr. with boiling carbon tetrachloride (250 c.c.). Excess of solvent was removed and the residual material washed free from fatty impurities with ether to give a mixture of sapogenins (4 g.), m. p. 200—250°. This mixture was chromatographed in benzene containing 20% of chloroform on a column of alumina. Elution with benzene–chloroform or chloroform gave, no sapogenin, but chloroform containing ½% of methanol eluted yuccagenin–agapanthagenin *complex* ($C_{54}H_{86}O_9$) (5—10% of the original mixture), which

[3] Whitby, *Biochem. J.*, 1923, **17**, 5; Schoenheimer, Dam, and von Gotberg, *J. Biol. Chem.*, 1953, **110**, 659.
[4] Spring and Swain, *J.*, 1943, 613; Plattner, Petrzilka, and Lang, *Helv. Chim. Acta*, 1944, **27**, 513.
[5] Dunn, Heilbron, Phipers, and Samant, *J.*, 1934, 1576.
[6] Petrow, Rosenheim, and Starling, *J.*, 1938, 677.

formed needles (from ethanol), m. p. 246—248°, $[\alpha]_D$ −171° (*c*, 1·6) (Found: C, 73·7; H, 9·8%; active hydrogen, 5. $C_{54}H_{86}O_9$ requires C, 73·8; H, 9·7%; active hydrogen, 5). Elution with chloroform containing increasing proportions of methanol furnished, after all the complex had been removed, small amounts of agapanthagenin (22a-spirostan-2α : 3β : 5α-triol); but this is so teniously held on alumina that elution is unsatisfactory. It can all be removed by treatment with boiling water, evaporation of the water, and extraction of the agapanthagenin with boiling carbon tetrachloride. *Agapanthagenin* crystallises from ethyl methyl ketone as plates, m. p. 285° (Found: C, 71·95; H, 10·0. $C_{27}H_{44}O_5$ requires C, 72·3; H, 9·8%). Light absorption in ethanol: inflections at 2050, 2100, and 2250 Å; ε 132, 59, and 18; it thus shows no region of maximal absorption associated with ethylenic centres. The specific rotation in chloroform and active hydrogen could not be determined because of its sparing solubility.

Agapanthagenin Diacetate (2α : 3β-*Diacetoxy*-22a-*spirostan*-5α-*ol*).—Agapanthagenin (2 g.) was acetylated with pyridine (7·5 c.c.) and acetic anhydride (3 c.c.); the *diacetate* (2 g.) crystallised from ethanol in needles, m. p. 298—299°, $[\alpha]_D$ −101° (*c*, 3·5) (Found: C, 70·0; H, 9·1; Ac, 16·1%; active hydrogen, 1. $C_{31}H_{48}O_7$ requires C, 69·9; H, 9·0; Ac, 16·1%; active hydrogen, 1). The infrared absorption spectrum in carbon disulphide showed the usual four bands, at 865, 900, 920, and 981 cm.$^{-1}$, the 900 band being stronger than the 920 as normally associated with the *iso*-configuration of ring F. The acetate band at 1244 cm.$^{-1}$ is typical of sapogenin diacetates and closely resembles that of gitogenin diacetate (2α : 3β-diacetoxy-5α : 22a-spirostan).[1] Light absorption in ethanol: inflections 2050, 2100, and 2250 Å; ε 410, 288, and 188. There was no region of maximal absorption associable with ethylenic centres.

Yuccagenin Diacetate (22a-*Spirost*-5-*en*-2α : 3β-*diol Diacetate*).—Acetylation of the agapanthagenin–yuccagenin complex (2 g.) with pyridine and acetic anhydride yielded a mixture (2 g.), m. p. 160—200°. Rapid fractional crystallisation of this from ethanol caused yuccagenin diacetate to separate first, and the agapanthagenin diacetate was purified separately (from ethanol) and found to be identical with that described above. The yuccagenin diacetate was crystallised from light petroleum (b. p. 40—60°), forming needles, m. p. 178°, $[\alpha]_D$ −130° (*c*, 2·8) (Calc. $[\alpha]_D$ −139°) (Found: C, 72·3; H, 9·0; Ac, 16·8. Calc. for $C_{31}H_{46}O_6$: C, 72·4; H, 8·9; Ac, 16·7%). The infrared absorption spectrum in carbon disulphide agreed with that recorded for yuccagenin diacetate.[1]

Chromatographic separation of the acetylated complex on alumina with light petroleum as solvent and 40% benzene–petroleum as eluant gave an *isomer* of yuccagenin diacetate. This crystallised from light petroleum as needles, m. p. 211°, $[\alpha]_D$ −112° (*c*, 2·5) (Found: C, 72·3; H, 8·85; Ac, 16·8. Calc. for $C_{31}H_{46}O_6$: C, 72·4; H, 8·9; Ac, 16·7%). The m. p. of a mixture with yuccagenin diacetate was 170—190°. Elution of the column with 10% of chloroform in benzene liberated agapanthagenin diacetate.

Yuccagenin. (22a-*Spirost*-5-*en*-2α : 3β-*diol*).—Yuccagenin diacetate was hydrolysed according to Perkin's procedure and yielded yuccagenin as needles (from ethanol), m. p. 248°, $[\alpha]_D$ −122° (*c*, 1·6) (Found: C, 75·4; H, 9·9. Calc. for $C_{27}H_{42}O_4$: C, 75·35; H, 9·8%).

Agapanthagenin–Yuccagenin Complex. ($C_{54}H_{86}O_9$).—Agapanthagenin or its diacetate was refluxed in 2N-alcoholic hydrochloric acid for 5 hr., the solution acquiring a deep greenish-yellow fluorescence. The complex produced crystallised from alcohol in needles, m. p. 246—248°, $[\alpha]_D$ −171° (*c*, 1·5) (Found: C, 73·7; H, 9·8%; active hydrogen, 5), and was identical with that obtained directly from the plant. It was similarly resolved on acetylation into yuccagenin and agapanthagenin diacetates.

Dehydration of Agapanthagenin Diacetate.—To agapanthagenin diacetate (2 g.) in dry pyridine (5 c.c.) at 20° thionyl chloride (1 c.c.) in pyridine (5 c.c.) was added dropwise with shaking. After 2 hr. pyridine hydrochloride had separated; dilute hydrochloric acid was added, and the product was extracted with ether. The ethereal solution was washed and dried, and the product crystallised several times from alcohol to give yuccagenin diacetate, identical with that previously obtained.

The author thanks Professor F. L. Warren and Professor H. Stephen for their interest.

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UNIVERSITY OF NATAL, HOWARD COLLEGE, DURBAN. [*Received, August 22nd,* 1955.]