Aust. J. Chem., 1975, **28**, 217–20

Chemical Examination of Local Plants. XIII* Elucidation of the Structure of a New Glycoside from the Leaves of Egyptian *Hibiscus sabdariffa*

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Abstract

The ether as well as the methanol extracts of the defatted leaves of *Hibiscus sabdariffa* yielded a new glycoside, the structure of which has been determined, by mass and n.m.r. spectra and by synthesis, as β-sitosteryl β-D-galactoside.

Hibiscus sabdariffa or karkade (fam. Malvaceae) is a medicinal plant and it is widely used in Egypt for therapeutic purposes, particularly for curing heart and nerve diseases.[1,2] Previously,[3] we reported the isolation of a new steroidal compound from the ether as well as from the methanol extracts of the defatted leaves of *H. sabdariffa*. The aim of the present work is to elucidate the structure of this compound.

The neutral part of the non-steam volatile fraction of the ether extract of the defatted leaves gave colourless rosettes. This material showed a broad band at ν_{max} 3450 cm^{-1} in the infrared spectrum, indicating the presence of a hydroxyl group. The n.m.r. spectrum showed one olefinic proton at δ 5·32 (triplet), corresponding to one trisubstituted double bond. The spectrum showed also a peak at δ 3·71 (triplet), probably due to a proton on the carbon vicinal to that carrying the hydroxyl group, and the complex splitting centred at δ 3·5 suggested that the compound did not have *gem*-dimethyl groups at C 4. The compound gave a green colour in the Liebermann–Burchardt test for steroids, and analysed for $C_{35}H_{60}O_6$.

The acetate of this alcohol had a molecular weight of 744 (by mass spectrum; the molecular ion M^+ was very weak and was detected with difficulty) which agrees with the molecular formula $C_{43}H_{68}O_{10}$. The spectrum showed also peaks at *m*/*e* 331, 229, 211, 169 and 109, which are characteristic for glycoside tetraacetate.[4] The peaks at *m*/*e* 414 and in particular that at 396 would seem to indicate the presence of derivatives of a $C_{29}H_{50}O$ alcohol. The n.m.r. spectrum showed four peaks at δ 2·1–2·0, corresponding to four acetate groups. The spectrum showed also a peak at δ 5·28 (triplet; one olefinic proton), indicating the presence of one trisubstituted double bond in the molecule. The peaks at δ 5·2–3·7 are characteristic of glycosidic

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* Part XII, *Egypt. J. Chem.*, in press.

[1] Gabriel, C., *Z. Unters. Lebensm.*, 1939, **77**, 561.

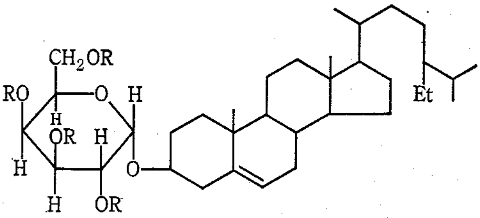
[2] Sharaf, A., *Planta Med.*, 1962, **10**, 48–52.

[3] Osman, A. M., El-Garby Younes, M., and Mokhtar, A., *Phytochemistry*, in press.

[4] McLean, J., personal communication.

protons adjacent to acetate groups.[4] Thus, from these results one can conclude that the glycoside may possess the partial formula $C_{29}H_{49}$–O–hexose.

Acid hydrolysis of the original tetrahydroxy compound yielded a steroidal aglycone of molecular weight 414, corresponding to the molecular formula $C_{29}H_{50}O$. The spectrum showed a peak at m/e 396 (20%), i.e. $M^+ - H_2O$. The n.m.r. spectrum of this aglycone showed a peak at δ 5·32 (one olefinic proton), confirming the presence of one trisubstituted double bond. The i.r. as well as the n.m.r. spectra of this aglycone are identical with those of authentic β-sitosterol.



(1a) R = H
(1b) R = OAc
(1c) R = OBz

The hexose sugar fraction obtained after acid hydrolysis was shown by paper chromatography as well as by t.l.c. to be galactose, which meant that the glycoside was β-sitosteryl galactoside.

This was confirmed, and the complete configuration determined, by synthesis of the glycoside from β-sitosterol and β-D-galactose, according to the procedure of Elderfield.[5] The synthesis was carried out by preparing galactose pentaacetate[6] from which acetobromogalactose was obtained which was then made to react with β-sitosterol.

From the above it follows that the glycoside is β-sitosteryl β-D-galactoside, and possesses the structural formula (1a). It is interesting to note that β-sitosteryl β-D-galactoside was not isolated from any natural source. Also, this galactoside was isolated from the methanol extract of the leaves.

Experimental

Specific rotations were determined in chloroform solutions unless otherwise stated. Infrared spectra of Nujol mulls were recorded on a Unicam SP200 G spectrometer, while nuclear magnetic resonance spectra were determined in deuterated pyridine or chloroform, using a Perkin–Elmer 40 MHz spectrometer, with tetramethylsilane as internal standard. Mass spectra were determined on an A.E.I. MS9 instrument. Thin-layer chromatography was conducted with plates coated with silica gel G (0·25 mm), and developed by placing the plates in an iodine tank for 15 min. Paper chromatography was run on Whatman No. 1 sheets using the ascending method, and the spots of the sugars were visualized by spraying the sheets with ethanolic benzidine and subsequent heating at 70° for 15 min. All melting points are uncorrected.

Isolation of β-Sitosteryl β-D-Galactoside

The defatted leaves (3·0 kg) were extracted with ether for 48 h and the extract (50 g) was steam-distilled. The non-volatile fraction (29·0 g) was hydrolysed with ethanolic potassium hydroxide (10%; 300 ml). The neutral portion (9·0 g) was dissolved in ether (50 ml) and left at room temperature overnight. Pale greenish yellow rosettes formed; they were filtered off and recrystallized thrice

[5] Elderfield, R. C., *J. Amer. Chem. Soc.*, 1947, **69**, 2235.
[6] Horning, E. C., *Org. Synth.*, 1955, Coll. Vol. III, 11.

from chloroform to give colourless rosettes of *β-sitosteryl β-*D*-galactoside* (1a) (0·5 g), m.p. 275–277° (dec.), $[\alpha]_D$ −63° (*c*, 0·52 in pyridine). ν_{max} 3450 cm^{-1} (broad; OH). N.m.r. in deuterated pyridine: δ 5·32 (triplet; one olefinic proton), 3·71 (triplet; proton α to hydroxyl group). The compound gave a green colour in the Liebermann–Burchardt test for steroids, and gave only one spot on a t.l.c. plate of R_F value 0·52 (MeOH–Et$_2$O 1 : 9) (Found: C, 72·7; H, 10·5. $C_{35}H_{60}O_6$ requires C, 72·9; H, 10·5%).

Acetylation

The galactoside (200 mg) in dry pyridine (1·5 ml) was heated with acetic anhydride (0·6 ml) at 100° for 2 h. Dilution with water followed by extraction with ether and working up through ether as usual gave *β-sitosteryl β-*D*-galactoside tetraacetate* (1b) (150 mg) as colourless needles (MeOH–CHCl$_3$), m.p. 149–151°, $[\alpha]_D$ −33·3° (*c*, 0·6). ν_{max} 1718 cm^{-1} (broad; OAc). The molecular weight by mass spectrum is 744 ($C_{43}H_{68}O_{10}$). The spectrum showed peaks at *m/e* 331, 229, 211, 169 and 109 and smaller peaks at *m/e* 414 and 396. The n.m.r. spectrum showed four sharp peaks at δ 2·1–2, which are due to four acetate groups, and at 5·28 (triplet; one olefinic proton), while a complex peak at δ 5·2–3·7 is due to proton adjacent to carbon atoms carrying the acetate groups (Found: C, 69·3; H, 9·1. $C_{43}H_{68}O_{10}$ requires C, 69·4; H, 9·1%).

Benzoylation

The galactoside (0·3 g) was benzoylated with benzoyl chloride (2 ml) and dry pyridine (2 ml) at 0° for 2 h. The reaction mixture was diluted with water and extracted with ether. Working up through ether as usual gave colourless plates of *β-sitosteryl β-*D*-galactoside tetrabenzoate* (1c) (0·2 g) (MeOH–CHCl$_3$), m.p. 196–198°, $[\alpha]_D$ +10·5° (*c*, 0·52), R_F value 0·7 (benzene) (Found: C, 76·6; H, 7·5. $C_{63}H_{76}O_{10}$ requires C, 76·2; H, 7·7%).

Acid Hydrolysis

The galactoside (0·23 g) was refluxed with ethanol (0·3 ml), water (0·3 ml) and concentrated hydrochloric acid (0·4 ml) for 1 h.[7] The prosapogenin was separated by filtration, and was refluxed further for 6 h with ethanol (6 ml), water (2 ml) and concentrated hydrochloric acid (2 ml). The cold reaction mixture was filtered off to give β-sitosterol (100 mg) as colourless plates (MeOH–CHCl$_3$), m.p. and m.m.p. 132°, $[\alpha]_D$ −34° (*c*, 0·52). The molecular weight by mass spectrum is 414, and the peak at *m/e* 396 (20%) is due to $M^+ - H_2O$; the R_F value of 0·47 (benzene–ether 4 : 1) is that of authentic β-sitosterol.

The filtrate from which the aglycone had been separated was shown by p.c. for sugar contents, using two solvent systems: (A) butanol : acetic acid : water (4 : 1 : 2·2 v/v); (B) butanol : acetic acid : water (4 : 1 : 5 v/v), to contain galactose of R_F values (0·12) and (0·11) respectively, while its R_F value on a t.l.c. plate is similar to that of authentic D-galactose (0·65), in solvent system (A) with ammoniacal silver nitrate as detecting agent.

*Synthesis of β-Sitosteryl β-*D*-Galactoside*

D-Galactose (6·6 g) was acetylated[6] with acetic anhydride (2·8 ml) and few drops of concentrated sulphuric acid for 2 h, followed by passing hydrogen bromide gas into the cooled mixture for 4 h. The mixture was allowed to stand at 5° overnight. Hydrogen bromide and acetic anhydride were removed by distillation under vacuum. The greenish gummy acetobromogalactose (6·7 g), which could not be induced to crystallize, was dissolved in dioxan (67 ml); 4 ml of this solution was poured dropwise over 1 h into a stirred mixture of β-sitosterol (200 mg), dry silver carbonate (175 mg),[5] magnesium sulphate (30 mg) and dry dioxan (4 ml). The mixture was allowed to react at room temperature for 20 h. The silver and magnesium salts were filtered off and the solvent was removed under vacuum. The crude product (200 mg) was chromatographed over alumina (10 g) in light petroleum. Elution with ether gave the pure β-sitosteryl β-D-galactoside tetraacetate (50 mg) as colourless prisms (MeOH–CHCl$_3$), m.p., m.m.p. 153–154°, $[\alpha]_D$ −30° (*c*, 0·9). The infrared spectra of the synthetic and the natural β-sitosteryl β-D-galactoside tetraacetate are superimposable.

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[7] Dugan, J. J., De Mayo, P., and Starratt, A. N., *Can. J. Chem.*, 1964, **42**, 491.

The β-sitosteryl β-D-galactoside from the methanol extract of the leaves was identical in all respects (i.r., m.p., m.m.p., specific rotation and R_F values) with the galactoside isolated from the ether extract above.

Acknowledgments

The authors would like to express their gratitude to Professor J. McLean and Dr P. Bladon of the University of Strathclyde, Glasgow, for the n.m.r. and mass spectral measurements.

Manuscript received 1 June 1974