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Octa(Per)-, Hepta- and Mono-Acetates of Dioscin[1]

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Acetylation of dioscin (I) with acetic anhydride–pyridine (1:1) at 10° for 24 hr gave, as the major product, a heptaacetate (III), mp 130—135°, $[\alpha]_D$ −82.0° (MeOH), $[\alpha]_D$ −85.3° ($CHCl_3$), in which one hydroxyl group, probably at C-3 of the glucose unit, is free.

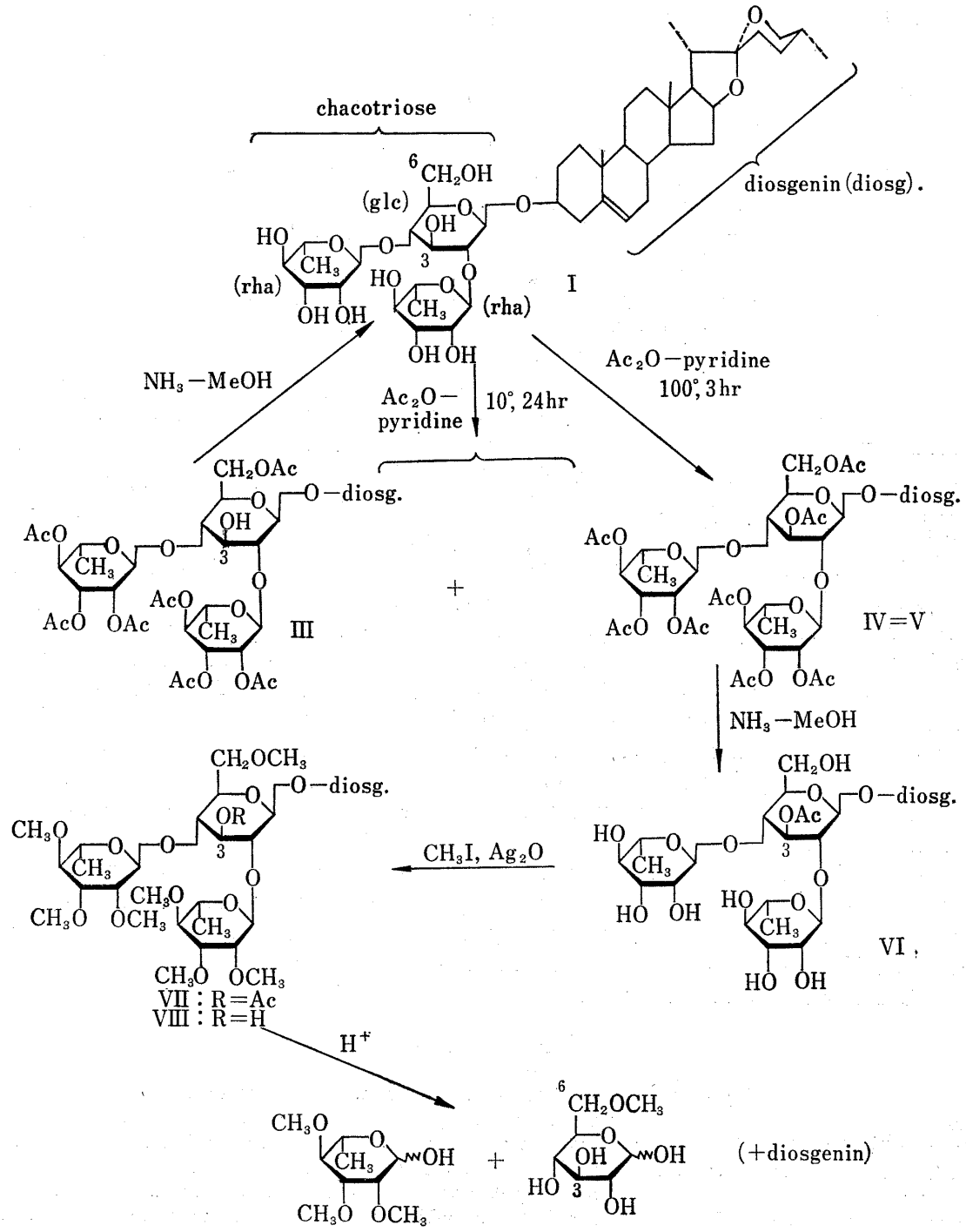
Dioscin octa(per)acetate (V), mp 145—147°, $[\alpha]_D$ −67.5° (MeOH), $[\alpha]_D$ −61.9° ($CHCl_3$), was obtained on treatment of I with acetic anhydride–pyridine (2:1) at 100° for 3 hr and subsequent purification of the product by recrystallization.

Ammonolysis of V provided a dioscin monoacetate (VI), mp 285—289° (decomp.), $[\alpha]_D$ −101.2° (pyridine), which was proved to be 2-O- and 4-O-bis-α-L-rhamnopyranosyl-(3-O-acetyl)-β-D-glucopyranoside of diosgenin.

Acetylation of dioscin (diosgenin 2-O- and 4-O-bis-α-L-rhamnopyranosyl-β-D-glucopyranoside=diosgenin β-chacotrioside) (I) with acetic anhydride and pyridine at room temperature and subsequent purification of the product by means of chromatography on alumina gave an acetate, mp 143—145°, $[\alpha]_D$ −46° ($CHCl_3$).[3] The acetate had been regarded on the basis of its analytical data and paper chromatographic behavior as the homogeneous octa(per)acetate of dioscin. Later, however, an examination with the aid of newly developed thin-layer chromatography indicated it to be a mixture of several compounds.[4] On the other hand, a related steroid saponin gracillin (diosgenin 2-O-α-L-rhamnopyranosyl-(3-O-β-D-glucopyranosyl)-β-D-glucopyranoside) was acetylated in the same manner and the crude acetate was purified by recrystallization without chromatography on alumina to give the nona(per)acetate (II), mp 205—207°, $[\alpha]_D$ −75° ($CHCl_3$),[5,6] which revealed a single spot on thin-layer[4] and no hydroxyl absorptions in the infrared spectrum. When II was passed through an alumina column the eluate showed on thin-layer the spot of II accompanied by those of more polar substances[4] suggesting that a saponin acetate is partially deacetylated by this procedure. The crude acetylation product of I was then directly recrystallized repeatedly but the expected homogeneous acetate was not obtained.

On treatment with acetic anhydride and pyridine at 10° for 24 hr I gave an acetate consisting mainly of two compounds, major one (III) and minor and less polar substance (IV). Thin-layer chromatographycally pure III was successfully isolated with the aid of column chromatography on silica gel as a crystalline powder, mp 130—135°, $[\alpha]_D$ −82.0° (MeOH),

1) Presented at the 86th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, October 22, 1966.
2) Location: *Katakasu, Fukuoka.*
3) T. Tsukamoto, T. Kawasaki, A. Naraki, and T. Yamauchi, *Yakugaku Zasshi*, **74**, 984 (1954); T. Tsukamoto, T. Kawasaki, and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **4**, 35 (1956).
4) T. Kawasaki and K. Miyahara, *Chem. Pharm. Bull.* (Tokyo), **11**, 1546 (1963).
5) T. Kawasaki, T. Yamauchi, and R. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **10**, 698 (1962).
6) The melting point and the rotation (mp 204°, $[\alpha]_D^{13}$ −47° (c=0.402, $CHCl_3$)) reported previously[7] for gracillin peracetate should be corrected. The sample prepared in the same way as ref. 3) and 4) and purified by repeated recrystallization had mp 210—210.5°, $[\alpha]_D^{21}$ −77.6° (c=0.96, $CHCl_3$) and showed the signals of nine acetyl groups in the NMR spectrum. NMR (τ, pyridine): 8.02 (3H), 7.97 (3H), 7.93 (9H), 7.77 (9H), 7.67 (3H) (CH_3COO).
7) T. Tsukamoto and T. Kawasaki, *Yakugaku Zasshi*, **74**, 1127 (1954); *Chem. Pharm. Bull.* (Tokyo), **4**, 104 (1956).



$[\alpha]_D$ −85.3° ($CHCl_3$). Taking the *Rf* value into account it was thought to be a dioscin acetate where probably one or two hydroxyl groups remained unacetylated. III was then trimethylsilylated and the nuclear magnetic resonance (NMR) spectrum of the ether indicated the presence of one trimethylsilyl group and seven acetyl functions to colloborate that III is a heptaacetate of dioscin.

When I was heated at 100° for 3 hr with acetic anhydride and pyridine and the product was recrystallized from acetone an acetate (V), mp 145—147°, $[\alpha]_D$ −67.5° (MeOH), $[\alpha]_D$ −61.9° ($CHCl_3$), was afforded. The acetate V was homogeneous and corresponded to IV on thin-layer and its NMR spectrum showed that it bears eight acetyl groups in the molecule. Therefore V is regarded as the pure octa(per)acetate of dioscin.

While treatment of III with methanolic ammonia regenerated I, V provided under the same condition a new compound (VI), mp 285—289° (decomp.), $[\alpha]_D$ −101.2° (pyridine). It was slightly less polar than I, analysed for $C_{47}H_{74}O_{17}\cdot 3H_2O$ and the NMR spectrum showed a signal of three protons due to acetyl group indicating VI to be a monoacetate of dioscin. VI was converted by Kuhn method[8] to a heptamethyl ether monoacetate (VII), mp 105—108°, $[\alpha]_D$ −90.7° (MeOH), (acetoxyl but no hydroxyl absorptions were observed in the infrared spectrum), and VII was subsequently hydrolyzed with alkali to provide a heptamethyl ether (VIII), mp 85—88°, $[\alpha]_D$ −101.1° (MeOH). Methanolysis of VIII followed by acid hydrolysis furnished a mixture of two methylated sugars which were separated by chromatography on charcoal–celite and identified as 6–O–methyl–D–glucose and 2,3,4–tri–O–methyl–L–rhamnose. In consequence VIII is the dioscin heptamethyl ether in which the hydroxyl group at C—3 of glucose residue is free and VII is the corresponding monoacetate.

Since VII was derived from VI, the latter is seemingly presumed to be the monoacetate where the acetoxyl group is located at C–3 of the glucose unit. But it is well known[9] that the facile acyl migration is encountered in partially acylated polyhydroxylic compounds such as sugars and cyclitols upon methylation with Purdie's reagent or by Kuhn method and, in general, on treatment under alkaline conditions, and hence the possibility that the acetoxyl group in VI had been located originally at C–6 of the glucose residue cannot be excluded. However, in partially acetylated aldose derivatives so far recorded in the literatures[9] an acetyl group is usually migrated away from C–1 and towards C–6 and invariably from a secondary to a primary hydroxyl group. Therefore the acetyl migration from C–6 to C–3 in the glucose unit which is glycosidically substituted at C–2 and C–4 is not considered to have proceeded and the dioscin monoacetate VI is defined as diosgenin 2–O–and 4–O–bis–α–L–rhamnopyranosyl–(3–O–acetyl)–β–D–glucopyranoside.

For the same reason as above, an acetyl migration during the partial ammonolysis of peracetate (V) to 3(glc)–O–monoacetate (VI) does not seem either to have taken place and the fact that V gave predominantly VI suggests the less susceptibility to ammonolysis of the acetate group at C–3 of the glucose residue compared with those at other positions of the sugar moiety. If it is assumed that the selectivity of ammonolysis is due to any steric effects to which the 3–substituent is subject, the hydroxyl group at the position concerned could also be presumed to be less reactive in acetylation than other seven hydroxyl groups and the heptaacetate III might be deemed to be the dioscin heptaacetate in which the hydroxyl group at C–3 of glucose residue is free.

Experimental[10]

Dioscin Heptaacetate (III)——Dioscin (I) (1 g) was dissolved in 10 ml each of Ac_2O and pyridine and the solution was left stand at 10° for 24 hr. The reaction mixture was poured into ice–water and the precipitates were collected by filtration, washed and dried: yield 1.16 g, *Rf* values 0.38 (major), 0.44. The product (800 mg) was placed on a column of silica gel (160 g) and eluted with benzene—acetone (10:1). Fractions which contained only the substance of *Rf* 0.38 were combined and evaporated to give a solid (327 mg), which was dissolved in MeOH and precipitated by adding water, collected by filtration and dried to

8) R. Kuhn, I. Löw, and H. Trischmann, *Chem. Ber.*, **88**, 1492, 1690 (1955).

9) S.J. Angyal and G.J.H. Melrose, *J. Chem. Soc.*, **1965**, 6494, 6501 and references cited therein; A. Lezerovich, E.G. Gros, J.F. Sproviero, and V. Deulofeu, *Carbohyd. Res.*, **4**, 1 (1967); I.A. Pearl and S.F. Darling, *Arch. Biochem. Biophys.*, **102**, 33 (1963); G. Entlicher and J. Kocourek, *Arch. Biochem. Biophys.*, **118**, 305 (1967).

10) Melting points were taken on a Kofler block and are uncorrected. IR spectra were obtained with a IR–S spectrophotomer (Japan Spectroscopic Co., Ltd.). NMR spectra were recorded at 60 Mcps with a JNM 3H–60 and C–60H spectrometers (Japan Electron Optics Lab. Co., Ltd.), tetramethylsilane being used as internal standard. Chemical shifts are given in τ units. *Rf* values were determined on thin–layer of silica gel G (Merck) using benzene—iso–PrOH (20:1) (for III and V) and $CHCl_3$–MeOH–water (7:3:1) (for I and VI and partially methylated sugars) as the solvents. In column chromatography "Kanto" silica gel (100—200 mesh) was employed.

provide III as a white powder, mp 130—135°, $[\alpha]_D^{32}$ −82.0° (c=0.51, MeOH), $[\alpha]_D^{20}$ −85.3° (c=0.70, $CHCl_3$). *Anal.* Calcd. for $C_{59}H_{86}O_{23}\cdot H_2O$ (dioscin heptaacetate monohydrate): C, 59.98; H, 7.51. Found: C, 60.11; H, 7.54. To the solution of III (200 mg) in pyridine (10 ml) were added hexamethyldisilazane (1 ml) and trimethylchlorosilane (10 drops) and the mixture was left stand at room temperature for 1 hr, evaporated *in vacuo* and the residue was extracted with benzene. The benzene solution was passed through a column of silica gel and the eluate was evaporated to give a solid (160 mg). IR ν_{max}^{Nujol} cm^{-1}: 1740 and 1235 (acetoxyl), no hydroxyl absorptions. NMR ($CDCl_3$): 9.85 (9H, $(CH_3)_3Si$–O–), 8.05 (3H), 8.03 (3H), 8.01 (3H), 7.99 (3H), 7.94 (3H) and 7.88 (6H) (CH_3COO).

Dioscin Octa(Per)acetate (V)——A solution of I (720 mg) in Ac_2O (10 ml) and pyridine (5 ml) was heated at 100° for 3 hr. The reaction mixture was poured into ice–water, the precipitates were collected by filtration and dried to give a powder (900 mg), which was recrystallized several times from acetone to afford V as colorless needles, mp 145—147°, $[\alpha]_D^{32}$ −67.5° (c=1.04, MeOH), $[\alpha]_D^{20}$ −61.9° (c=1.23, $CHCl_3$), *Rf* 0.44. *Anal.* Calcd. for $C_{61}H_{88}O_{24}\cdot H_2O$ (dioscin octaacetate monohydrate): C, 59.89; H, 7.42. Found: C, 59.95; H, 7.37. NMR ($CDCl_3$): 7.97 (6H), 7.93 (3H), 7.90 (3H), 7.85 (3H) and 7.82 (9H) (CH_3COO).

Dioscin Monoacetate (VI)——Octaacetate (V) (900 mg) in 100 ml MeOH saturated with ammonia was left stand at room temperature for 24 hr. The solution was diluted with water and extracted with BuOH saturated with water. The BuOH layer was washed with water and evaporated *in vacuo* to dryness. The residue was crystallized[11] from MeOH to give VI as colorless needles (550 mg, *Rf* 0.32, I 0.28). Analytical sample was obtained by repeated recrystallization from MeOH as fine needles, mp 285—289° (decomp.), $[\alpha]_D^{29}$ −101.2° (c=1.1, pyridine). IR ν_{max}^{Nujol} cm^{-1}: 1745 and 1220 (acetoxyl). NMR (pyridine): 7.78 (3H, CH_3COO). *Anal.* Calcd. for $C_{47}H_{74}O_{17}\cdot 3H_2O$ (dioscin monoacetate trihydrate): C, 58.49; H, 8.36. Found: C, 58.44; H, 8.32.

Ammonolysis of heptaacetate (III) (50 mg) with 3 ml of MeOH saturated with ammonia in the same way as for V gave I as needles, mp 270—275° (decomp.), *Rf* 0.28.

Heptamethyldioscin Monoacetate (VII)——To the solution of VI (690 mg) in dimethylformamide (10 ml) were added CH_3I (5 ml) and Ag_2O (5 g) and the mixture was stirred at room temperature for 24 hr. The precipitates were removed by filtration, CH_3I (3 ml) and Ag_2O (3 g) were added to the filtrate and the mixture was stirred for additional 24 hr. The reaction mixture was worked up according to the Kuhn's procedure[8] and the crude product (565 mg) was chromatographed on silica gel using benzene–acetone (5:1) as a solvent. The eluate was evaporated to dryness and the residue (507 mg) was precipitated from MeOH with water to give VII as a crystalline powder, mp 105—108°, $[\alpha]_D^{28}$ −90.7° (c=0.89, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1760 and 1225 (acetoxyl), no hydroxyl absorptions.

Heptamethyldioscin (VIII)——Methylether monoacetate (VII) (415 mg) was dissolved in 2% KOH in MeOH (50 ml) and the solution was refluxed for 1 hr. A residue, obtained on removal of the solvent *in vacuo*, was treated with water and the insoluble substance was collected by filtration and precipitated from MeOH with water to provide VIII as a powder (150 mg), mp 85—88°, $[\alpha]_D^{28}$ −101.1° (c=0.33, MeOH). *Anal.* Calcd. for $C_{52}H_{86}O_{16}\cdot H_2O$ (dioscin heptamethyl ether monohydrate): C, 63.39; H, 9.00. Found: C, 63.35; H, 9.03. The solution of VIII (480 mg) in 7% HCl in MeOH (20 ml) was refluxed for 3 hr, MeOH was removed *in vacuo* and the residue was heated at 100° with 1 N HCl (10 ml) for 1 hr. The reaction mixture was neutralized with Ag_2CO_3, the precipitates were filtered off and the filtrate was evaporated *in vacuo* to give a syrup (95 mg). It revealed two spots (*Rf* 0.19, 0.82) on thin-layer chromatograms. The syrup was dissolved in 5% EtOH, placed on a column (20 mm × 160 mm) of charcoal–celite (1: 2) and eluted with 5% EtOH: Fr. 1 (200 ml), *Rf* 0.19, 25 mg; Fr. 2 (300 ml), trace; Fr. 3 (80 ml), *Rf* 0.82, 31 mg. Fr. 1 was crystallized form EtOH to give a needle, mp 132—138°, *Rf* 0.19 (6–O–methyl–D–glucose[12]: mp 143—146°, *Rf* 0.19; 3–O–methyl–D–glucose[13]: mp 157—158°, *Rf* 0.13).

Fr. 3 and Fr. 1 were converted into methyl glycoside and methyl glycoside trimethylsilyl ether, respectively, and examined by gas–liquid chromatography (GC–550F Gas Chromatograph equipped with a hydrogen flame ionization detector (Yanagimoto Mfg. Co., Ltd), glass column 1.2 m long, 2 mm ϕ, packed with 5% 1,4–butanediol succinate[14] on Shimalite W (60—80 mesh)). Retention time (min): methyl glycoside of Fr. 3, 1.7 (methyl 2,3,4–tri–O–methyl–L–rhamnoside, 1.7: N_2 flow rate 22 ml/min; flash heater temp. 205°, column temp. 122°, detector temp. 185°); methyl glycoside trimethylsilyl ether of Fr. 1, 3.9 (methyl 3–O–methyl glucoside trimethylsilylate 1.6, methyl 6–O–methyl glucoside trimethylsilylate 3.9: N_2 flow rate 53 ml/min; flash heater temp. 210°, column temp. 122°, detector temp. 210°).

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11) The residue (216 mg) obtained from the mother liquor consisted of VI and minor amount of I.
12) Prepared according to H.R. Wood, Jr., *et al.*, (*J. Am. Chem. Soc.*, **79**, 3862 (1957)).
13) Prepared according to G.A. Anderson, *et al.*, (*J. Chem. Soc.*, **1929**, 1329).
14) G.O. Aspinall, *J. Chem. Soc.*, **1963**, 1676.