SAPONINS FROM THE SEEDS OF *COSTUS SPECIOSUS*[1]

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ABSTRACT.—Studies of *Costus speciosus* seeds have afforded ten steroidal saponins. These have been isolated and their structures elucidated as β-sitosterol-β-D-glucopyranoside, prosapogenin-B of dioscin, prosapogenin-A of dioscin, dioscin, gracillin, 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl], 26-O-[β-D-glucopyranosyl]-22α-methoxy-(25R)-furost-5-en-3β,26-diol, methyl protodioscin, and protodioscin.

Diosgenin is extensively used as a raw material for the synthesis of important drugs such as corticosteroids and oral contraceptives. The rhizomes of *Costus speciosus* (Koen.) Sm. are well known for their diosgenin content and also for several saponins (1, 2). Recently, we have reported the seeds of this plant as an additional source of diosgenin (3). Since no record is available of the saponins of the seeds, we wish to report our findings from a study of the saponins of the seeds.

RESULTS AND DISCUSSION

The dried, powdered and defatted seeds were extracted with methanol. The methanol extracts, on further defatting and extraction with *n*-butanol, yielded a mixture of saponins. Tlc examination of this mixture showed the presence of at least ten spots. The crude saponin mixture was chromatographed on silica gel column and eluted with chloroform containing varying proportions of methanol.

All the compounds thus obtained gave positive tests (frothing, red blood corpuscle hemolysis and a positive Liebermann-Burchard test) for saponins. The first five (low yield) were negative to Ehrlich reagent, while the last five (higher yield) were positive, indicating the latter set as furostanol saponins. The ir spectrum of all the compounds, except one, showed absorptions at approximately 980, 920, 900 and 860 cm^{-1} characteristic of steroidal saponins (4).

The configuration of the sugars was determined by anomeric hydrogen coupling constants and by Klyne's rule of molecular rotation (5). The structures were further proved by periodate oxidation and Smith degradation (6, 7) and also by direct comparison (mmp, co-tlc, co-ir) with their authentic samples.

Besides, β-sitosterol-β-D-glucopyranoside, the spirostanol saponins which have been identified were prosapogenin-B of dioscin (diosgenin-3-O-α-L-rhamnopyranosyl (1→4)-β-D-glucopyranoside), prosapogenin-A of dioscin (diosgenin-3-O-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside), dioscin (diosgenin-3-O-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranoside) and gracillin (diosgenin-3-O-α-L-rhamnopyranosyl (1→2) [β-D-glucopyranosyl (1→3)-β-D-glucopyranoside.

The furostanol saponins were identified as 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl)-26-O-[β-D-glucopyranosyl]-22α-methoxy (25R)-furost-5-en-3β,26-diol, methyl protodioscin (3-O-(α-L-rhamnopyranosyl (1→4) [α-L-rhamnopyranosyl (1→2)]-β-D-glucopyranosyl) 26-O-[β-D-glucopyranosyl]-22-α-methoxy-(25R)-furost-5-en-3β,26-diol), and protodioscin (3-O-(α-L-rhamnopyranosyl (1→4) [α-L-rhamnopyranosyl (1→2)]-β-D-glucopyranosyl) 26-O-[β,D,glucopyranosyl]-(25R)-furost-5-en-3β,22α,26-triol).

3-O-furostanol-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl-26-O-[β-D-glucopyranosyl]-22-α-methoxyl (25R)-furost-5-en-3, 26-diol on complete acid-hydrolysis, afforded diosgenin (44.8%), D-glucose and L-rhamnose. The quantitative hydrolysis and colorimetric estimation (8, 9) of sugars revealed the ratio 1:2:1 for diosgenin, D-glucose and L-rhamnose, respectively. Partial hydrolysis of the same saponin furnished prosaponins which were identified as trillin (dios-

[1]Part II in the series "Plant Saponins". Part I see *Planta Med.*, **40**(3), 301 (1980).

genin-3-O-β-D-glucopyranoside) and prosapogenin of dioscin. The latter and D-glucose were also obtained by enzymatic hydrolysis with almond emulsin. These results suggested that the furostanol was probably 3-O-[α-L-rhamnopyanosyl (1→2)-β-D-glucopyranosyl]-(25R)-22α-methoxy-furost-5-en-3β,26-diol, a substance previously reported in *Trillium kamtschaticum* (10).

This structure was confirmed by the following: the presence of a methoxyl resonance at δ 3.05 and anomeric proton resonance in the ^{1}H nmr spectrum of the furostanol saponin and its methyl ether, respectively; ms studies on the deca-acetate, hydrolysis of the methyl ether [2,3,4-tri-O-methyl-L-rhamnose (R_G 1.01), 2,3,4,6-tetra-O-methyl-D-glucose (1.00) and 3,4,6-tri-O-methyl-D-glucose (0.86)] and Baeyer-Villiger Oxidation (11–13) of the deca-acetate to methyl-γ-methyl-δ-hydroxy pentanoate-β-D-glucopyranoside, tetraacetate and 5α-pregnan-3β,5α,6β, 16β,20α-pentanol tetraacetate.

METHYL PROTODIOSCIN.—The ir spectrum and the positive Ehrlich reaction showed methyl protodioscin to be a furostanol saponin. Acid hydrolysis afforded diosgenin, D-glucose and L-rhamnose in 1:2:2 molar ratio. Partial and enzymatic (almond emulsin) hydrolysis of methyl protodioscin afforded dioscin and D-glucose. The latter and trillin were obtained on periodate oxidation and Smith degradation, respectively. These results suggested that methyl protodioscin might be 3-O-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl]-26-O-[β-D-glucopyranosyl]-(25R)-22α-methoxy-furost-5-en-3β,26-diol, which is known from *Dioscorea spetemloba* (14). This structure was finally confirmed by the presence of a methoxyl singlet at δ 3.05 in methyl protodioscin and anomeric proton resonance in the ^{1}H nmr spectrum of its methyl ether, hydrolysis of the methyl ether [2,3,4-tri-O-methyl-L-rhamnose (1.01), 2,3,4,6-tetra-O-methyl-D-glucose (1.00) and 3,6-di-O-methyl-D-glucose (0.56)], ms studies of its dodeca-acetate (15) and Baeyer-Villiger oxidation of the dodeca-acetate to methyl-γ-methyl-δ-hydroxypentanoate-β-D-glucopyranoside tetraacetate and 5α-pregnan-3β,5α,6β,16β,20α-pentanol tetraacetate.

PROTODIOSCIN.—The Ehrlich test and ir spectrum of Protodioscin indicated it to be a furostanol saponin. Acid hydrolysis afforded diosgenin, D-glucose and L-rhamnose in 1:2:2 molar ratio. Partial and enzymatic hydrolysis of protodioscin furnished dioscin as methyl protodiscin did. These reactions suggested that protodioscin might have a 22-hydroxy group instead of the 22-methoxy group as in methyl protodioscin. Therefore, the structure of protodioscin conforms to 3-O-(α-L-rhamnopyranosyl (1→4) [α-L-rhamnopyranosyl (1→2)]β-D-glucopyranosyl)-26-O-[β-D-glucopyranosyl]-(25R)-furost-5-en-3β,22α,26-triol which was reported earlier from *Tribulus terrestris* (16).

The ^{1}H nmr spectrum of neither protodioscin nor its acetate displayed any signal for methoxy protons. Further protodioscin, on boiling with methanol, was converted into methyl protodioscin; methyl protodioscin was converted into protodioscin on boiling with aqueous acetone. This type of interconversion of hydroxy/methoxy furostanol saponins was reported by Tschesche *et al.* (17). Thus it was concluded that protodioscin is a furostanol saponin of methyl protodioscin having an OH group at C–22 in place of OCH_3 group.

EXPERIMENTAL[2]

[2]Mps are uncorrected; ir: Perkin Elmer 177, 157 and 566; ^{1}H-nmr: Perkin Elmer R–32 (90 MHz) or Varian A–60 D (60 MHz) in $CDCl_3$, internal reference TMS; chemical shift δ (ppm); ms: JEOL–JMS–D–300 with JMA–200 data-processor; tlc: Silica gel-G (BDH) and compounds visualized with Ehrlich reagent and/or 10% H_2SO_4; CC: Silica-gel (60–120 mesh; BDH); Descending pc on Whatmann No. 1 paper; and aniline hydrogen phthalate was used as developer. All *Rf* values are calculated from tlc unless otherwise stated. All R_G values are reported with respect to 2,3,4,6-tetra-O-methyl-D-glucose on pc. Different solvent systems used for pc are: (a) *n*-butanol-acetic acid-water (4:1:5); (b) *n*-butanol-aniline-water (6:4:3), (c) *n*-butanol-ethanol-water (4:1:5).

All the acetylations were done with acetic anhydride and pyridine, while permethylation was done by Hakomori's method [18].

ISOLATION OF SAPONINS.—The air-dried and powdered seeds (2.37 kg) of *C. speciosus* (Koen.) Sm. collected[3] from the farms of this Institute were defatted with hexane (5 liters) for 16 hr. in a Soxhlet. The defatted material was then extracted with methanol (5 liters) for 16 hr., and the extract was freed from the solvent. The viscous mass (160 g) was taken up in water (1.2 liters) and partitioned successively between hexane, chloroform and ether to remove traces of the fats and oils. Finally, the aqueous layer was extracted with *n*-butanol saturated with water (5 x 300 ml). The butanol extract was repeatedly washed with water and the solvent removed under reduced pressure. This furnished a crude mixture of saponins (110 g). This crude mixture exhibited at least 10 spots on tlc. The spots with lower Rf values were very close and in higher concentrations. The crude saponin mixture (66 g) was chromatographed over Si gel (1.5 kg) and eluted with chloroform with varying percentages of methanol, which yielded pure saponins.

β-Sitosterol-β-D-glucopyranoside.—The cc fractions 8–14 eluted with chloroform-methanol, (85:15) on crystallization from methanol, yielded microneedles (0.16 g, 0.01%), mp 280–2° (decomp).

PROSAPOGENIN B OF DIOSCIN.—Prosapogenin of dioscin was obtained as colorless crystals (0.10 g, 0.006%) from methanol from the cc fractions 15–24 eluted with 15% methanol-chloroform. It gave mp 250–5° (decomp); ir: ν max 3600–3200, 1180–1000, 980, 960, 920(w), 900(s) and 870 cm^{-1}. Found: C, 64.26; H, 8.37; $C_{39}H_{62}O_{12}$ requires C, 64.81; H, 8.58%. Hexamethyl ether obtained as semisolid, ^{1}H-nmr (90 MHz, CCl_4), 4.05 (1H, d, J=7 Hz) 4.7 (1H, brs, $W½$= 5 Hz).

PROSAPOGENIN A OF DIOSCIN.—Prosapogenin A of dioscin was crystallized from methanol as a hydroscopic powder (0.036 g, 0.002%) from cc fractions 37–55, mp 200–5° (decomp); ir: ν max 3600–3200, 1180–1000, 980, 965, 920(w), 900(s) and 860 cm^{-1}. Found: C, 62.95; H, 8.67, $C_{39}H_{62}O_{12}$. H_2O; requires C, 63.24; H, 8.64%. Hexamethyl ether: homogeneous syrup, ^{1}H-nmr (60 MHz, CCl_4), 4.05 (1H, d, J=7 Hz), 4.8 (1H, brs, W½=5 Hz).

DIOSCIN.—Crystallization of fractions 56–105 from methanol furnished colorless crystals (0.50 g, 0.003%), mp 286–7° (decomp); ir: ν max 3650–3200, 1200–1000, 980, 960, 920(w), 900(s), 865 and 840 cm^{-1}. Found C, 60.05, H, 8.65; $C_{45}H_{72}O_{16}$. [H_2O requires C, 60.94, H, 8.35%. Octamethyl ether, mp 119–21°; ^{1}H-nmr (90 MHz, CCl_4); 4.15 (1H, d, J=7.5 Hz), 4.65 (1H, brs, W½=4.5 Hz), 4.92 (1H, brs, W½=5 Hz). ms: M/e (rel. int. %), 567(8.1), 449(100), 413(5.0), 399(1.7), 397(30.0), 396(12.6), 189(100), 139(19.1). Found: C, 64.70; H, 9.30; $C_{53}H_{88}O_{16}$ requires C, 64.79; H, 8.98%. Acetate, mp 140–1°; ir ν max 1745, 1240, 980, 960, 920(w), 900(s), 860 and 840 cm^{-1}. ^{1}H-nmr (90 MHz, $CDCl_3$), 4.45 (1H, d, J=7.5 Hz), 4.65 (1H, brs, W½=4.5 Hz), 4.70 (1H, brs, W½=5 Hz).

GRACILLIN.—Gracillin, obtained from cc fractions 134–166, was crystallized from methanol (0.132 g, 0.008%), mp 296–300° (decomp.); ir: ν max 3600–3100, 1190–1000, 982, 960, 918(w), 898(s), 860 and 845 cm^{-1}. Found C, 59.16, H, 8.16; $C_{45}H_{74}O_{17}$. [H_2O requires C, 59.87, H, 8.20%. Nonamethyl ether: Homogeneous syrup. ^{1}H-nmr (90 MHz, CCl_4), 4.05 (2H, d, J=7.5 Hz), 4.80 (1H, brs, W½=5 Hz). Acetate: mp 201–4°; ir: ν max 1740, 1360, 1220, 980, 920(w), 900(s), 840 cm^{-1}; ^{1}H-nmr (90 MHz, CCl_4), 4.3 (1H, d, J=8.5 Hz), 4.48 (1H, d, J=7 Hz), 4.70 (1H, brs, W½=5 Hz); ms: m/e (rel. int. %) 618(17.3), 604(74.0), 477(100), 413(23.0), 331(11.8), 273(17.6), 242(24.8), 157(100), 139(11.0), 115(100).

3-O-FUROSTANOL-α-L-RHAMNOPYRANOSYL (1→2) β-D-GLUCOPYRANOSYL-26-O-[β-D-GLUCOPYRANOSYL] 22-α-METHOXYL-(25R)-FUROST-5-EN-3,26-DIOL.—Eluates 207–256 from the column, after concentration and crystallization from methanol-acetone, afforded the saponin (2.00 g, 0.12%), mp 245–9° (decomp.); ir: ν max 3650–3100, 1200–1000, 980, 920(s), 900(w) and 845 cm^{-1}; ^{1}H-nmr (DMSO-d_6) 1.5 (3H, brs, CH_3 of rha), 3.05 (3H, S, OCH_3), 4.3 (2H, d, J=7 Hz), 5.0 (1H, brs, W½=5 Hz). Found: 57.0; H, 8.26; $C_{46}H_{76}O_{18}$. [$2H_2O$ requires C, 57.98; H, 8.40%. Decamethylether: homogenous syrup. ^{1}H-nmr (CCl_4) 4.05 (2H, d, J=7 Hz), 4 92 (1H, brs, W½=4.5 Hz). Acetate: mp 160–2°; ir: ν max 1740 and 1220 cm^{-1}; ms: m/e (rel. int. %) 1304 (M^+−32, 0.2 1031(.5), 973(.25), 775.(6), 744(4.5), 726(66.6), 561(10.0), 511(8.0), 396(35.5), 331(100), 273(100).

METHYL PROTODIOSCIN.—Cc fractions 282–359 eluted with 20% methanol-chloroform, on crystallization from methanol, afforded shining crystals (3.26 g, 0.20%), mp 192–6° (decomp.); ir: ν max 3700–3050, 1130–1000, 980, 960, 920(s), 900(w), and 840 cm^{-1}; ^{1}H-nmr (DMSO-d_6) 1.4 (6H, CH_3 of rha), 3.05 (3H, S, OCH_3), 4.65 (2H, brs, W½=7 Hz), 5.0 (2H, brs, W½=5 Hz). Found: C, 56.05; H, 8.36; $C_{52}H_{86}O_{22}$. [$2H_2O$ requires C, 56.83; H, 8.20%. Dodecamethylether, amorphous powder mp 91–93°; ^{1}H-nmr (CCl_4) 4.15 (1H, d, J=7 Hz), 4.75 (1H, brs, W½=4 Hz), 4.90 (1H, brs, W½=4 Hz). Found: C, 62.45; H, 8.73; $C_{64}H_{110}O_{22}$ requires C, 62.44; H, 8.94%. Acetate: mp 130–31°; ir: ν max 1750, 1220, 980, 910, 890 and 840 cm^{-1}; ms: m/e (rel. int. %), 1304(1.5), 1262(.2), 1204(.35), 915(.75), 791(1.2), 744(1.3), 726(46.8), 552(37.9), 511(10.0), 510 (15.0), 331(100), 273(100). Found: C, 58.18; H, 7.14; $C_{76}H_{110}O_{34}$ requires C, 58.24; H, 7.02%.

PROTODIOSCIN.—Protodioscin was obtained as a colorless powder (1.0 g, 0.06%) from aqueous methanol from cc fractions 360–379, mp 267–71° (decomp.); ir: ν max 3600–3100, 1150–1000, 980, 918(s), 898(w) and 840 cm^{-1}; ^{1}H-nmr (60 MHz, DMSO-d_6), 4.60 (2H, brs, W½=7.5 Hz), 4.95 (2H, brs, W½=4.5 Hz). Found: C, 56.15; H, 8.15; $C_{51}H_{84}O_{22}$. $2H_2O$ requires C, 56.46; H, 8.12%. Acetate: mp 180–1°; ir: ν max 3500, 1740, 1220, 980, 910(s), 895(w) and 840 cm^{-1}. Found: C, 57.91; H, 6.83; $C_{75}H_{105}O_{34}$ requires C, 57.99; H, 6.96%.

[3]A voucher specimen of the seeds has been deposited in the Herbarium of CIMAP.

HYDROLYSIS OF SAPONINS.—All saponins were separately refluxed with 7% methanolic sulfuric acid on a water bath for 6–7 hrs. The reaction mixture, on removal of methanol and subsequent extraction with chloroform, afforded a common genin, disogenin. The aqueous layer, after neutralization by passing through Dowex-3 and concentration, afforded D-glucose and L-rhamnose (varying ratio), which were identified by co-pc (system a, b) and estimated colorimetrically (8, 9).

PARTIAL HYDROLYSIS OF SAPONINS.—Each saponin was refluxed with 1% methanolic sulfuric acid for 25 min. The reaction mixture was diluted with water and extracted with *n*-butanol. The *n*-butanol, on concentration, furnished a prosapogenin mixture, which was resolved by chromatography over Si gel, eluted with mixtures of chloroform and methanol; pure prosapogenins were obtained.

HYDROLYSIS OF PERMETHYLATED SAPONINS AND IDENTIFICATION OF METHYLATED SUGARS.—The permethylated saponins were hydrolyzed by heating with Kiliani mixture for approximately 5 hr. The methylated sugars thus obtained were identified (6b, 19) by their R_G values and also by comparison with authentic samples by paper chromatography (system C).

Each methylated sugar was subjected to periodic acid oxidation. Except for 4,6-di-O-methyl-D-glucose, none was effected. The reported R_G value of 3–6-di-O-methyl-D-glucose was lower than the observed value. The observed value compared favorably with 2,3-di-O-methyl-D-glucose (R_G 0.57) and 3,4-di-O-methyl-D-glucose (R_G 0.52), but the periodate oxidation and Smith degradation of dioscin and HIO_4 oxidation of methylated sugars ruled out the possibility of either of these two.

ENZYMATIC HYDROLYSIS OF SAPONINS.—The saponins, gracillin (10 mg), the furostanol-2,26-diol-3-O-glycoside (50 mg), methyl protodioscin (200 mg) and protodioscin (50 mg) were incubated separately with almond emulsin at 42° for 7 days. After the usual work up, the first two saponins afforded prosapogenins, which were identified as prosapogenin A of dioscin, while methyl protodioscin and protodioscin afforded dioscin.

CONVERSION OF METHYL PROTODIOSCIN INTO PROTODIOSCIN AND VICE VERSA.—Methyl protodioscin (50 mg) was boiled with aqueous acetone (50 ml) on a water bath for 18 hr. and the solvent was removed to furnish protodioscin (co-tlc, mmp). Protodioscin (55 mg) was boiled with methanol (30 ml) for 24 hr on a water bath. The product was concentrated and crystallized from methanol to afford methyl protodioscin (co-tlc, mmp).

BAEYER-VILLIGER OXIDATION OF 3-O-FUROSTANOL-α-L-RHAMNOPYRANOSYL (1→2) β-D-GLUCOPYRANOSYL-26-O-[β-D-GLUCOPYRANOSYL]-22α-METHOXY-(25R)-FUROST-5-EN-3,26-DIOL.—A solution of the furostanol 22-methoxy-3,26-diol-glycoside (530 mg) in dichloroethane (20 ml) was heated to 50° with formic acid (90%, 20 ml) and hydrogen peroxide (30%, 2.2 ml). The reaction mixture, on evaporation *in vacuo* and alkaline hydrolysis (methanolic KOH, 3%, 30 ml) followed by acetylation and cc on silica-gel with benzene-chloroform (1:1), afforded two fractions.

The first fraction, a colorless oil, ^{1}H-nmr (90 MHz) $CDCl_3$), 0.91 (3H, d, J=8 Hz CH–CH_3), 3.65 (3H, *S*, OCH_3) 4.48 (1H, d, J=7 Hz, C_1H of glu); ms *m/e* 417 (M^+-CH_3COO), 331, 243, 242, 200, 169, 157, 149, 145, 140, 129, 115, 109, 103, 98 and 97 was identified as methyl-γ-methyl-δ-hydroxy-pentanoate-β-D-glucopyranoside tetraacetate (I) (20). D-glucose was identified from this product when it was treated with NH_3 in methanol and subsequent enzymatic hydrolysis (almond emulsin).

The second fraction, on trituration with ether-hexane, yielded an amorphous powder (II, 210 mg), mp 168–70° of which 200 mg on subsequent hydrolysis with 5% methanol-HCl followed by acetylation (Ac_2O–C_5H_5N) afforded a compound, mp 214–16° $[\alpha]_D-25°$ ($CHCl_3$), which was identified as 5α-pregnan-3β,5β,6β,16β,20α-pentanol-tetraacetate (III, 50 mg) (21) by comparison with an authentic sample.

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LITERATURE CITED

1. B. Dasgupta and V. B. Pandey, *Experientia*, **26**, 475 (1970).
2. V. B. Pandey and R. Tschesche, *Phytochemistry*, **17**, 1971 (1978).
3. S. B. Singh, M. M. Gupta, R. N. Lal and R. S. Thakur, *Planta Med.*, **38**, 185 (1980).
4. L. F. Fieser and M. Fieser, "Steroids" Reinhold, New York, 1959 pp 832.
5. W. Klyne, *Biochem. J.*, **47**, xLi (1950).
6. F. Smith and R. Montgomery, "The chemistry of Plant Gums and Mucilages," Reinhold Publishing Corporation, New York, 1959 (a) pp. 195 (b) pp. 225.
7. A. K. Chakravarty, C. R. Saha and S. C. Pakrashi, *Phytochemistry*, **18**, 902 (1979).
8. S. B. Misra and V. K. Mohan Rao, *J. Sci. Ind. Res.*, **19c**, 173 (1960).
9. R. B. Singh and K. P. Tewari, *Phytochemistry*, **17**, 1991 (1978).
10. T. Nohara, K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.*, **23**, 872 (1975).
11. R. E. Marker and D. L. Turner, *J. Am. Chem. Soc.*, **62**, 2540 (1940).
12. K. Morita, S. Noguchi, H. Kono and T. Miki, *Chem. Pharm. Bull.*, **11**, 90, 144 (1963).
13. S. B. Mohato, N. P. Sahu, B. C. Paland and R. N. Chakravarti, *Ind. J. Chem.*, **15B**, 445 (1977).

14. S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, T. Hosokawa and T. Kawasaki, *Chem. Pharm. Bull.*, **16**, 1162 (1968).
15. T. Komori, Y. Ida, Y. Mutou, K. Miayahara, T. Nohara and T. Kawasaki, *Biomed. Mass Spectorm*, **2**, 65 (1975).
16. M. Tomoba and R. Gyulemetova, *Planta Med.*, **34**, 188 (1978).
17. R. Tschesche and G. Wulff, Fortschritte der Chemie Organischer Naturstoffe". Vol. 30, edited by W. Herz, H. Grisebach & G. Kirby, Springer-Verlag, Wien, New York, 1973, pp. 486.
18. S. Hakomori, *J. Biochem.* **55**, 205 (1964).
19. E. Lederer and M. Lederer, "Chromatography", Elsevier Publishing Company, Amsterdam, 1957, pp. 249.
20. R. Tschesche, G. Ludke and G. Wulff, *Chem. Ber.*, **102**, 1253 (1969).
21. T. Miki, K. Morita, S. Naguchi, T. Kishi, K. Hiraga, and H. Nawa, *Chem. Pharm. Bull.*, **11**, 95 (1963).