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STEROIDAL SAPONINS OF *COSTUS SPECIOSUS**

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Key Word Index—*Costus speciosus*; Costaceae; gracillin; dioscin; diosgenin content.

Abstract—The structures of the saponins B and C have been established as gracillin and dioscin respectively. This is the first report of the occurrence of these saponins in the family Costaceae.

INTRODUCTION

Various pharmacological activities have been reported [2, 3] for the rhizomes of *C. speciosus*, a common plant distributed throughout India. The isolation of tigogenin, diosgenin, saponin A (sitosterol-glycoside) and saponins B, C and D has been reported previously [4–6] from the rhizomes of the plant. The rhizomes of the plant have also been found [7] to be a rich source of diosgenin. This paper describes the structural elucidation of saponins B and C.

RESULTS AND DISCUSSION

The total saponin mixture isolated from the methanolic extract of the dried, as well as fresh rhizomes showed the presence of five saponins on TLC, all were negative to Ehrlich reagent [8, 9]. It indicated the absence of any furostanol saponins. CC and Si gel gave the previously reported saponins A (sitosterol-glycoside), B, C and a mixture of two minor saponins. On acid hydrolysis, both B and C yielded diosgenin, glucose and rhamnose. GLC analysis of the persilylated methyl glycoside of the sugars from B and C showed the ratios of glucose and rhamnose to be 2:1 and 1:2, respectively.

Saponin B. The type of the glycosidic linkage in B was proved by methylation. The permethylated product after hydrolysis and chromatography on Si gel gave 2,3,4-tri-*O*-methyl-L-rhamnose(**1**), 2,3,4,6-tetra-*O*-methyl-D-glucose(**2**) and 4,6-di-*O*-methyl-D-glucose(**3**), which were identified by direct comparison and the anilide derivatives with authentic specimens. In addition the GC–MS studies of the partially methylated alditol acetates from the sugars of B showed the presence of **1**, **2**, **3** in the ratio of 1:1:1. The sequence of the sugars in B was proved by partial hydrolysis with HCl, which gave a monoglycoside(**4**) and diglycoside(**5**) and a trace of diosgenin. Glucose and rhamnose were identified in the hydrolysate. Acid hydrolysis of **4** gave only glucose whereas **5** gave glucose and rhamnose in the ratio of 1:1. Glycoside **4** and **5** were next methylated and after hydrolysis with HCl, **4** gave 2,3,4,6-tetra-*O*-methyl-D-glucose(**2**) and **5** gave 2,3,4-tri-*O*-methyl-L-rhamnose(**1**) and 3,4,6-tri-*O*-methyl-D-glucose(**6**). GC–MS studies of the alditol acetates from **5** showed the presence of **1** and **6** in the ratio of 1:1. The above results are in excellent agreement with gracillin [diosgenin α-L-rhamnopyranosyl($1 \rightarrow 2_{Glc,1}$)-β-D-glucopyranosyl($1 \rightarrow 3_{Glc,1}$)-β-D-glucopyranoside] and in fact it was found to be indistinguishable from gracillin by direct comparison (mp, mmp, TLC, IR).

Saponin C. The permethylated product of C, after hydrolysis and CC on Si gel gave 2,3,4-*O*-methyl-L-rhamnose(**1**) and 3,6-di-*O*-methyl-D-glucose(**7**). Compound **7** was identified by direct comparison with authentic material. GC–MS studies of the methylated alditol acetates of C showed the presence of **1** and **7** in the ratio of 2:1. Partial hydrolysis gave monoglycoside **4** and diglycoside **5** and a trace of diosgenin. Rhamnose was identified in the acid hydrolysate. The above results are in close agreement with dioscin [diosgenin bis-α-L-rhamnopyranosyl($1 \rightarrow 2$ and $1 \rightarrow 4$)-β-D-glucopyranoside] and in fact it was found to be indistinguishable from dioscin by direct comparison (mp, mmp, TLC, IR).

Gracillin and dioscin were first isolated by Tsukamoto *et al.* [10] and Honda [11] respectively from *Dioscorea* species and their structures were established by Kawasaki and Yamauchi [12]. This is the first report of the isolation of gracillin and dioscin from a plant of the family Costaceae. The reinvestigation of the diosgenin content in the methanolic extract of the dried rhizomes yielded 2.41% diosgenin. It shows that *C. speciosus* may be utilised as a convenient [5–7] commercial source for the isolation of diosgenin.

EXPERIMENTAL

Mps are uncorr. GLC was on a column (2 m) containing 3% OV-101 on Gas Chrom Q with N_2 (30 ml/min) at 155°. GC–MS were carried out using a glass column (200 × 0.2 cm) containing 3% OV-225 on Gas Chrom Q at 180°. TLC was on Si gel G (Merck) using Ehrlich reagent and 10% H_2SO_4 as detector. PC was on Whatman No. 1 using aniline hydrogen phthalate for staining. CC was on Si gel (0.05–0.1 mm, Fa. Hermann, Köln). The following solvents were used for CC, TLC and PC: A, $CHCl_3$–MeOH–H_2O (65:20:10, organic phase + 10% MeOH); B, $CHCl_3$–MeOH–H_2O (65:35:10, lower phase) [13]; C,

* Part 14 in the series "Steroidal Saponins of the Spirostanol Type". For part 13 see ref. [1].

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$CHCl_3$–EtOH (7:3); D, C_6H_6–Me_2CO (10:1); E, C_6H_6–Me_2CO (4:1); F, EtOAc–Py–H_2O (3:6:1:1.15, organic phase) [14]; G, *Iso*-octane–*iso*-PrOH 10% aq. NH_3 (65:25:2).

Isolation of saponins. Air dried rhizomes (1.72 kg) collected during August from the Botanical Garden, Institute of Medical Sciences, Banaras Hindu University, Varanasi City, India, were extracted with 90% MeOH at room temp. The MeOH extract (215 g) was taken in H_2O, defatted with C_6H_6 and extracted with BuOH. The BuOH extract exhibited a mixture of 5 saponins on TLC (system C), all were negative to Ehrlich reagent. The saponin mixture was applied to a Si gel column and eluted with system A to give: saponin A (sitosterol glycoside), mp 289–90°; saponin B(gracillin), mp 298–302°, $[\alpha]_D^{20}$ −86.2 (DMF, c = 0.12) (Found: C, 59.16, 58.82; H, 8.16, 7.95%), acetate mp 208°, $[\alpha]_D^{20}$ −45 ($CHCl_3$, c = 0.392); saponin C (dioscin), mp 288–90°, $[\alpha]_D^{20}$ −110.1 (DMF, c = 0.14) (Found: C, 59.75, 60.18; H, 8.71, 8.34%) and a mixture of two minor saponins. IR (in KBr) of both B and C showed characteristic absorption bands for spirostanol saponins [15, 16].

Hydrolysis of B and C. 50 mg of B or C were hydrolysed with 2 N HCl (dioxan–H_2O, 1:1, 25 ml) for 8 hr. Diosgenin was obtained from both B and C, mp 208–09°, M⁺ 414 (mmp, superimposable IR and NMR with authentic sample). Glucose and rhamnose were identified in the hydrolysate from both the saponins by PC (system F). Quantitative determination [17] of sugars was carried out by GLC of the silyl derivatives which showed that the proportions of glucose and rhamnose were 2:1 for B and 1:2 for C.

Methylation and hydrolysis of the methylated B and C. B (650 mg) was methylated by Hakomori's method [18]. The permethylated product (342 mg), IR (in KBr) on OH band, was refluxed first with 5% methanolic HCl for 6 hr and then with 2 N HCl at 100° for 4 hr. After neutralisation with Dowex-3, it was chromatographed over Si gel (40 g) with system D and E to yield 2,3,4-tri-*O*-methyl-L-rhamnose (**1**, 35 mg syrup), $[\alpha]_D^{20}$ +27° (H_2O, c = 0.53), anilide, mp 121–23°; 2,3,4,6-tetra-*O*-methyl-D-glucose (**2**, 12 mg), mp 90–92° (petrol), $[\alpha]_D^{20}$ +76.2° (H_2O, c = 1.01) and 4,6-di-*O*-methyl-D-glucose (**3**), mp 160–63° (EtOAc), $[\alpha]_D^{20}$ +0.110 → +64° (H_2O, c = 0.95). Methylation and hydrolysis of the methylated C after eluting on Si gel gave **1** and 3,6-di-*O*-methyl-D-glucose (**7**), mp 113–117° (EtOAc), $[\alpha]_D^{20}$ +58.6° (H_2O, c = 0.195). Compounds **1**, **2**, **3** and **7** were identified by direct comparison, mmp, TLC (system B), PC (system G) with authentic specimens.

Partially methylated alditol acetates from B and C. Methylated alditol acetates [19–21] from B and C were prepared after methylation, hydrolysis of methylated products, reductions with $NaBH_4$ and subsequent acetylation. GC–MS studies of the alditol acetates showed the presence of **1**, **2** and **3** in the proportion of 1:1:1 in B whereas **1** and **7** were in the proportion of 2:1 in C.

Partial hydrolysis of B and C. B or C was dissolved in dioxan and heated with 0.15 N HCl at 90° for 6 hr. Dioxan was removed and extracted with BuOH. The BuOH extract was chromatographed over a Si gel column with system A. B furnished monoglycoside (**4**), mp 195–97°, $[\alpha]_D^{20}$ −84.2° (DMF, c = 0.194) and diglycoside (**5**), mp 229–35°, $[\alpha]_D^{20}$ −88.6° (DMF, c = 0.12). C gave monoglycoside (**8**) and diglycoside (**9**) which were identified to be identical with **4** and **5** respectively (by methylation, hydrolysis of the methylated products, mmp, TLC, PC).

Methylation and hydrolysis of the methylated **4** *and* **5**. Methylation and hydrolysis of **4** gave compound **2**. Methylation and hydrolysis of **5** showed a mixture of two compounds, which were applied to a Si gel column and eluted with system E to give compound **1** and 3,4,6-tri-*O*-methyl-D-glucose (**6**). GC–MS of the alditol acetates of **5** showed **1** and **6** in the proportion of 1:1. **6** was also identified by TLC (system B) and PC (system G) with an authentic sample.

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