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COSTUSOSIDE-I AND COSTUSOSIDE-J, TWO NEW FUROSTANOL SAPONINS FROM THE SEEDS OF *COSTUS SPECIOSUS**

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Key Word Index—*Costus speciosus*; Costaceae; furostanol saponin; 3-*O*-{β-D-glucopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl)-(25*R*)-furost-5-en-3β, 22α, 26-triol and its 22α-methoxy derivative.

Abstract—The structure of costusoside I and costusoside J have been established as 3-*O*-{β-D-glucopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2) [α-L-rhamnopyranosyl (1→4)]-β-D-glucopyranosyl}-26-*O*-(β-D-glucopyranosyl-22α-methoxy-25*R*)-furost-5-en-3β, 26-diol and its 22-hydroxy compound respectively, isolated from the seeds of *Costus speciosus*.

INTRODUCTION

Costus speciosus seeds (Costaceae) have already been reported as a source of diosgenin [1]. In a continuation of this investigation, we have isolated a variety of saponins from the methanolic extract of mature seeds of this plant [2]. The present paper deals with the structure elucidation of two new furostanol saponins designated as costusoside I and costusoside J from the *n*-butanol fraction of the methanolic extract of the seeds.

RESULTS AND DISCUSSION

The *n*-butanol fraction of the methanolic extract of the seeds of *C. speciosus* showed the presence of ten saponins on TLC which have been designated as compounds A–J in increasing order of their polarity. The first five (low yield) were negative to Ehrlich reagent while the last five (higher yield) were positive, thus indicating the former set to be spirostanol and the latter to be furostanol saponins. The mixture of saponins was resolved on a Si gel column and compounds A–H identified as 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 - (25*R*) - furost - 5 - en - 3β,26 - diol, methyl protodioscin and protodioscin respectively [2]. Compounds I (costusoside I) and J (costusoside J) were new compounds and were subjected to structure elucidation studies.

Costusoside I (**1a**) $C_{58}H_{96}O_{27}$, mp 220–224°, $[\alpha]_D$ − 76.5°, exhibited a pink colour with Ehrlich reagent. The IR spectrum of **1a** showed absorptions at 980, 915, 895 and 840 cm^{-1} besides the hydroxyl and ether bands characteristic of steroidal saponins. The band at 915 was stronger than that at 895 cm^{-1} confirming it to be a furostanol saponin [3].

Complete acid hydrolysis of **1a** afforded diosgenin (35%), D-glucose and L-rhamnose in the molar ratios 1 : 3 : 2 respectively. The sugars were estimated by colorimetric estimation [4, 5].

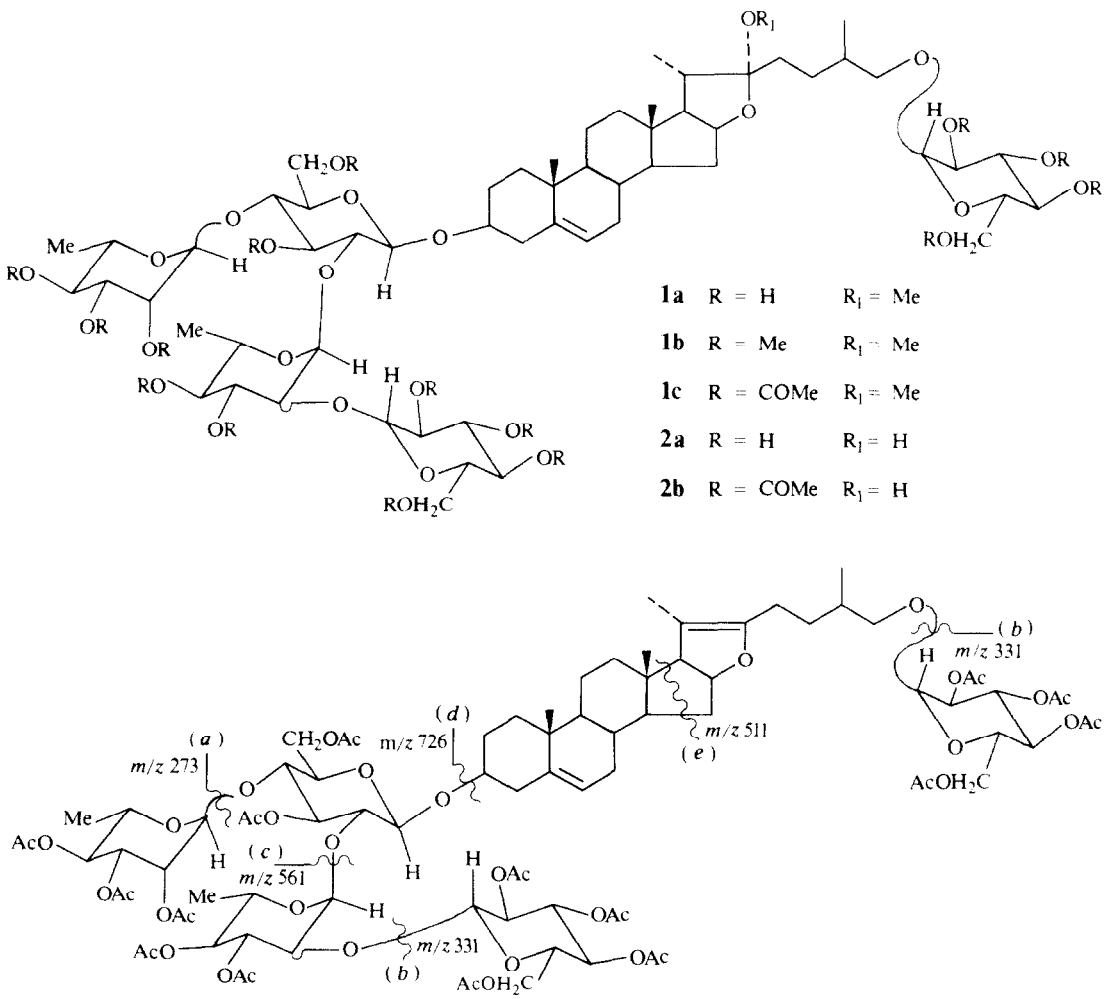
Enzymatic hydrolysis of **1a** with almond emulsin produced initially a prosaponin identified as methyl protodioscin (3 - *O* - {α - L - rhamnopyranosyl(1→4)[α - L - rhamnopyranosyl(1→2)] - β - D - glucopyranosyl} 26 - *O* - (β - D - glucopyranosyl) - 22α - methoxy - (25*R*) - furost - 5 - en - 3β,26 diol) and finally dioscin (diosgenin - 3 - *O* - α - L - rhamnopyranosyl(1→4)[α - L - rhamnopyranosyl(1→2)] - β - D - glucopyranoside) and D-glucose indicating that it was a higher glycoside of the same basic structure as methyl protodioscin and dioscin. This result suggested that another glucose unit was present as an end sugar to methyl protodioscin.

Compound **1a** on partial hydrolysis afforded methyl protodioscin, dioscin and a prosaponin PSI. Prosaponin PSI on enzymatic hydrolysis yielded prosapogenin A of dioscin and D-glucose. The former was also obtained by further partial hydrolysis of PSI. These results suggested that the other glucose was attached through the rhamnose of prosapogenin A of dioscin [diosgenin - 3 - *O* - α - L - rhamnopyranosyl(1→2) - β - D - glucopyranoside].

Permethylation of **1a** by Hakomori's method [7] afforded a pentadecamethyl ether (**1b**) which on hydrolysis furnished 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-L-rhamnose and 3,6-di-*O*-methyl-D-glucose. The branched sugar linkage of glucose at C-3 of diosgenin was established by periodate oxidation and Smith degradation [2, 6] which yielded diosgenin, D-glucose and trillin respectively.

The ^1H NMR spectra of **1a** and its pentadecaacetate (**1c**) displayed singlets at δ 3.10 and 3.58 respectively, possibly for a methoxy group at C-22 of methyl protodioscin. The mass spectrum of **1c** showed prominent peaks at *m/z* 273 (triacetyl rhamnose), 331 (tetra-acetyl glucose), 561 (peracetylated rhamnosyl glucose), 726 and 511 by cleavage of **1d** at

*Part 3 in the series "Plant Saponins". For Part 2, see ref. [2].

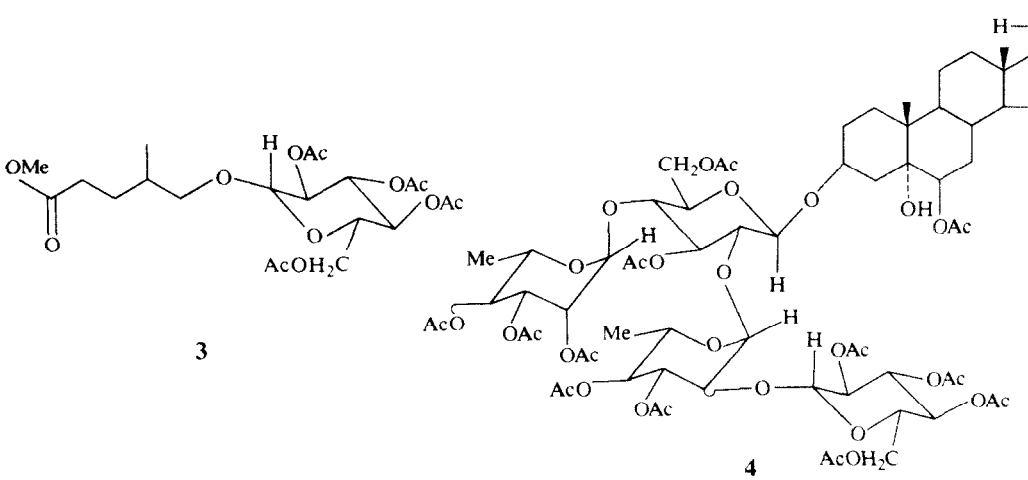


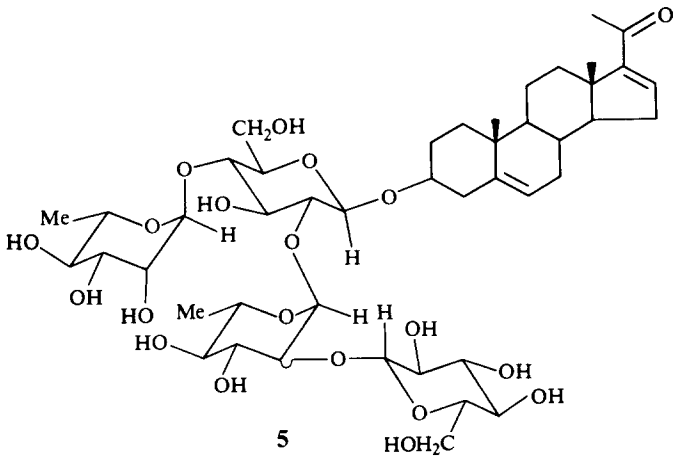
Scheme 1. Mass spectral fragmentation of **1d** [**1e** – MeOH].

a, *b*, *c*, *d* and *e* respectively like other peracetylated furostanol saponins (Scheme 1). This fragmentation confirmed the presence of glucose at C-26 of diosgenin.

The presence of one glucose at C-26 and a methoxy group at C-22 was establised by the Baeyer–Villiger oxidation [8, 9] of **1c** followed by alkaline hydrolysis and subsequent acetylation which yielded methyl - γ - methyl - δ - hydroxypentanoate - β - D - glucopyranoside tetra-acetate (**3**) and an acetylated glycoside (**4**) which on acid hydrolysis furnished 5α - pregnan - 3β,5α,6β,16β,20α - pentanol tetra-acetate.

The ^{1}H NMR spectrum of **1b** displayed three doublets (1H each) at δ 4.10, 4.25 and 4.40 with J = 7, 7.5 and 6.5 Hz respectively attributable to anomeric pro-





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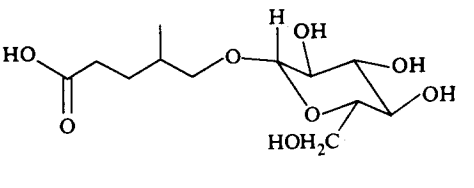

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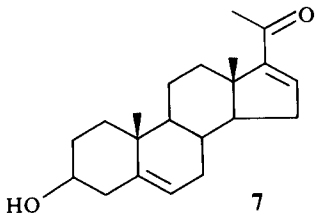

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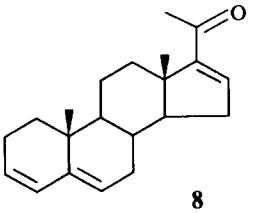

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tons of glucose in the β-configuration and a broad singlet of two protons at δ 5.1 ($W_{1/2} = 5$ Hz) to anomeric protons of rhamnoses in the α-configuration. Similar results were obtained for **1a** (see Experimental). These configurations were also evident from the results of enzymatic hydrolysis and application of Klyne's rule [10] of molecular rotation.

On the basis of the above findings the structure of costusoside I was assigned as 3 - *O* - {β - D - glucopyranosyl(1→2) - α - L - rhamnopyranosyl(1→2)[α - L - rhamnopyranosyl(1→4)] - β - D - glucopyranosyl}, 26 - *O* - (β - D - glucopyranosyl) - 22α - methoxy - (25*R*) - furost - 5 - en - 3β,26 - diol (**1a**).

Costusoside J (**2a**) $C_{57}H_{94}O_{27}$, mp 248–250°, $[\alpha]_D$ −79.1° gave a positive test with Ehrlich reagent and showed absorptions typical of furostanol saponins in its IR spectrum.

The ^{1}H NMR spectra of **2a** and its acetate (**2b**) did not display any signal for methoxy protons. The rest of the spectra were comparable to the spectra of **1a** and **1c** respectively. This indicated that both compounds differed only in respect of one methoxy group. The mass spectrum of the acetate (**2b**) was very similar to that of **1c**.

Substance **2a** when boiled with methanol was converted into **1a*** which on boiling with aqueous acetone was converted into **2a**. Such interconversions are possible between 22-hydroxy and 22-methoxy furostanol glycosides [11]. Thus **2a** seemed to be a 22-hydroxy analogue of **1a**. This was ascertained by the following observations.

The acetate **2b** on boiling with glacial acetic acid formed a pseudoacetate due to the loss of the hydroxyl at C-22 in the form of H_2O. Treatment of the resultant dehydro compound with chromic acid in acetic acid resulted in a ring E cleavage product which on treatment with alkali furnished two products, glycoside **5** and the side-chain containing carboxylic acid **6**. Compound **6** was identified by esterification and subsequent acetylation which afforded methyl - γ - methyl - δ - hydroxy pentanoate - β - D-glucospyranoside tetra-acetate. While the glycoside (**5**) on acid hydrolysis afforded 3 β-hydroxy-pregna-5, 16-dien-20-one (**7**), pregna-3,5,16-trien-20-one (**8**), D-glucose and L-rhamnose.

Consequently, the structure of costusoside J was established as 3 - *O* - {β - D - glucopyranosyl(1→2) - α - L - rhamnopyranosyl(1→2)[α - L - rhamnopyranosyl(1→4)] - β - D - glucopyranosyl}, 26 - *O* - (β - D - glucopyranosyl) - (25*R*) - furost - 5 - en - 3β,22α,26 triol (**2a**).

*This suggested that **1a** may be an artefact.

EXPERIMENTAL

Mps were determined in open capillaries and were uncorr. IR spectra were recorded in KBr. The ^{1}H NMR spectra were recorded in $CDCl_3$ unless otherwise stated using TMS as internal reference. TLC was performed on Si gel-G and spots visualized with Ehrlich reagent and/or 10% H_2SO_4. Si gel (60–120 mesh, BDH) was used for CC. Descending PC were done on Whatmann No. 1 paper and aniline hydrogen phthalate was used for developing. All R_f values refer to TLC (Si gel-G) 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 TLC, CC or PC are: (A) $CHCl_3$–MeOH–H_2O (10 : 5 : 1); (B) $^-CHCl_3$–MeOH–H_2O (35 : 15 : 3); (C) C_6H_6–Me_2CO (17 : 3); (D) C_6H_6–Me_2CO (4 : 1); (E) $CHCl_3$–Me_2CO (19 : 3); (F) C_6H_{12}–EtOAc (4 : 1); (G) *n*-BuOH–HOAc–H_2O (4 : 1 : 5); (H) *n*-BuOH–pyridine–H_2O (6 : 4 : 3); (I) *n*-BuOH–EtOH–H_2O (4 : 1 : 5).

Isolation of saponins. Costusoside I and costusoside J were isolated and purified from *Costus speciosus* (Koen.) Sm. seeds as described previously [2].

Costusoside I. Crystallized from MeOH as shining crystals (5.9 g, 0.36%), mp 220–224° (decomp.), $[\alpha]_D^{25} - 76.5°$ (pyridine; *c* 1.52), $[M]_D - 936.36$ (obs.), $[M]_D - 955$ [Calc. for 3 (β-D-glucose) + 2 (α-L-rhamnose)], R_f 0.39 (System A). IR ν_{max}^{KBr} cm^{-1}: 3600–3100, 1120–1000, 980, 915(s), 895(w), 840. ^1H NMR (60 MHz, DMSO-d_6): δ 3.10 (3H, *s*, OCH_3), 4.10 (1H, *br s*, $W_{1/2} = 7$ Hz), 4.20 (1H, *br s*, $W_{1/2} = 7.5$ Hz), 4.3 (1H, *br s*, $W_{1/2} = 6.5$ Hz), 4.8 (1H, *br s*, $W_{1/2} = 5$ Hz), 5.0 (1H, *br s*, $W_{1/2} = 4.5$ Hz), 5.25 (1H), *br s*, H-6). (Found : C, 56.3; H, 7.40. $C_{58}H_{96}O_{27} \cdot 2H_2O$ requires: C, 56.04; H, 7.89%).

Costusoside J. Crystallized from MeOH as colourless crystals (0.6 g, 0.036%), mp 248–250° (decomp.).$[\alpha]_D^{25} - 79.1°$ (pyridine; *c* 1.32), $[M]_D - 957.11$ (obs.), $[M]_D - 955$ [Calc. for 3 (β-D-glucose) + 2 (α-L-rhamnose)] R_f 0.37 (System A). IR ν_{max}^{KBr} cm^{-1}: 3800–3100, 1180–990, 980, 910(s), 895(w), 840. ^1H NMR (60 MHz, DMSO-d_6): δ 4.12 (1H, *br s*, $W_{1/2} = 7$ Hz), 4.25 (1H, *br s*, $W_{1/2} = 7$ Hz), 4.32 (1H, *br s*, $W_{1/2} = 7$ Hz), 4.75 (1H, *br s*, $W_{1/2} = 5$ Hz), 5.0 (1H, *br s*, $W_{1/2} = 5$ Hz), 5.25 (1H, *m*, H-6). (Found: C, 55.54; H, 7.50. $C_{57}H_{94}O_{27} \cdot H_2O$: requires: C, 55.70; H, 7.81%).

Acid hydrolysis of costusoside I (**1a**) *and J* (**2a**). Costusoside I (62.2 mg) and J (60 mg) were each refluxed with 7% methanolic H_2SO_4 (20 ml) for 7 hr. Each soln was diluted with an equal vol. of H_2O and heated for 1 hr. MeOH was removed under red. pres. and the remaining material again diluted with H_2O. The reaction mixture was extracted with $CHCl_3$, dried over dry Na_2SO_4 and solvent removed under vacuum. Each residue afforded genin (21.8 mg, 35%) and **2a** (21.6 mg, 36%) identified as diosgenin by direct comparison (TLC, mmp, IR, NMR, MS) with an authentic sample. The hydrolysate was neutralized by passing through Dowex-3 (OH^- form) resin and concd under red. pres. This concentrate furnished D-glucose and L-rhamnose identified by PC (System G, H). The sugars were estimated colorimetrically [4, 5] and the ratio of D-glucose to L-rhamnose was found to be 3 : 2 respectively in both compounds.

Partial hydrolysis of **1a**. Compound **1a** (1.0 g) was refluxed with 1% methanolic H_2SO_4 for 25 min. The reaction mixture was diluted with H_2O and extracted with *n*-butanol. The *n*-butanol extract on concn furnished a mixture of prosaponins which were separated by chromatography over Si gel, eluting with a mixture of $CHCl_3$ and MeOH (10–15%) afforded prosaponin methyl protodioscin, PSI and dioscin. The first and last compounds were identified by TLC, mp, mmp and IR with an authentic sample. Prosaponin PSI on further partial hydrolysis furnished a prosaponin which was identified as prosapogenin-A of dioscin by direct comparison with an authentic sample.

Permethylation of costusoside I. Costusoside I (500 mg) was permethylated by Hakomori's method [7]. The reaction afforded crude methyl ether which was purified by chromatography on Si gel (12 g) elution with solvent system C gave pentadecamethyl ether (**1b**, 200 mg) mp 96–97°, R_f 0.48 (System D), the IR spectrum showed no absorption for a hydroxy group. (Found: C, 61.80; H, 8.75. $C_{73}H_{126}O_{27}$ requires: C, 61.60; H, 8.94%).

Hydrolysis of **1b**. Compound **1b** (85 mg) was hydrolysed with Kiliani mixture (20 ml) for 5.5 hr. The methylated sugars on PC in system I exhibited four spots with R_G values 1.01, 1.00, 0.84 and 0.56 and these were identified as 2,3,4-tri-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-L-rhamnose and 3,6-di-*O*-methyl-D-glucose respectively [12, 13]. The first two were confirmed by direct comparison with authentic samples while the last two were recognized by their R_G values and periodate oxidation results. The 3,6-di-*O*-methyl-D-glucose was also confirmed by comparison with an authentic sample obtained by permethylation and hydrolysis of dioscin.

Enzymatic hydrolysis of costusoside I and J and prosaponin PSI. Costusoside I (500 mg) and J (20 mg) were each incubated with almond emulsin for 7 days at 37°. The progress of both reactions was monitored by TLC. The TLC (System B) of the reaction mixture of **1a** initially showed the presence of methyl protodioscin while **2a** yielded protodioscin and finally both compounds were converted into dioscin. The dioscin was obtained after completion of the reaction and its identity was confirmed by comparison (TLC, mmp) with an authentic sample.

Similarly enzymatic hydrolysis of prosaponin PSI produced prosapogenin A of dioscin which was identified by direct comparison.

Acetylation of costusoside I and J. Costusoside I (1.0 g) was acetylated with Ac_2O–pyridine (5 ml each) and worked-up in the usual way. It afforded a pentadeca-acetyl derivative (**1c**, 959 mg), mp 99–100° R_f 0.55 (System B). IR ν_{max}^{KBr} cm^{-1}: 1750, 1230, 980, 910(s), 890(w) and 840. MS *m/z* (rel. int.) 726 (0.13), 561 (0.12), 519 (0.19), 511 (0.11), 413 (0.46), 331 (2.1), 273 (1.92), 211 (8.04), 149 (100), 139 (16.8). (Found: C, 56.95; H, 7.05. $C_{88}H_{126}O_{42}$ requires: C, 56.96; H, 6.79%).

Costusoside J (500 mg) was similarly acetylated to obtain a pentadeca-acetate (**2b**) mp 105–106°, R_f 0.40 (System E), IR ν_{max}^{KBr} cm^{-1}: 3500 (broad, OH), 1745, 1240, 1040, 980, 919(s), 890(w) and 840. MS *m/z* (rel. int.). 726 (3.56), 561 (1.3), 511 (0.3), 413 (0.42), 331 (28.3), 273 (54.6), 211 (11.4), 149 (100), 139 (12.8). (Found C, 56.83, H, 6.56. $C_{87}H_{124}O_{42}$ requires: C, 56.74; H, 6.74%).

Baeyer–Velliger oxidation of **1c**. Compound **1c** (605 mg) in dichloroethane (20 ml), formic acid (90%, 20 ml) and H_2O_2 (30%, 2.2 ml) was heated at 50° in an oil bath for 30 min and then distilled *in vacuo*. The crude residue was hydrolysed with methanolic KOH (3%, 20 ml) by warming it at 50° for 30 min. It was then cooled and brought to pH 7 by adding dil. HCl. The solvent was removed under vacuum and the resulting product was acetylated with Ac_2O–pyridine (6 ml each) by heating on a water bath for 2.5 hr. A mixture of the two products thus obtained was chromatographed on Si gel (15 g). Elution with C_6H_6–$CHCl_3$ (1 : 1) gave a colourless oil which was identified by ^1H NMR and MS as methyl-γ-methyl-δ-hydroxy pentanoate-β-D-glucopyranoside tetra-acetate (**3**) reported by Tschesche *et al.* [14]. Its MS had peaks at *m/z* 417 $[M - MeCOO]^+$, 331, 243, 242, 200, 169, 157, 149, 145, 140, 129, 115, 109, 103, 98 and 97. ^1H NMR (90 MHz): δ 0.91 (3H, *d*, $J = 8$ Hz, $\rangle$CH–Me), 3.65 (3H, *s*, OMe), 4.48 (1H, *d*, $J = 7$ Hz, anomeric H of glucose). This product was treated with NH_3 in MeOH on a water bath for 15 min and then allowed to stand at room temp. for 1 hr. Thereafter the reaction mixture was concd *in vacuo*. The residue thus obtained was hydrolysed with almond emulsin to yield glucose (PC).

The crude solid obtained from the second fraction of the chromatography was crystallized from *n*-hexane–Et_2O which afforded an amorphous powder (**4**, 320 mg), mp 135–137°, R_f 0.50 (System D), IR ν_{max}^{KBr} cm^{-1}: 3500, 1735, 1360, 1220, 1040. Compound **4** (250 mg) on hydrolysis with 5% methanolic HCl and subsequent acetylation of the aglycone with Ac_2O–pyridine and purification by crystallization from a mixture of *n*-hexane–Et_2O gave 5α-pregnan-3β, 5α,6β,16β,20α-pentanol tetra-acetate.

Conversion of **1a** *to* **2a** *and vice-versa.* The costusoside I (100 mg) was refluxed with aq. Me_2CO (40 ml) for 18 hr. The solvent was removed and the residue crystallized from aq.

MeOH to yield compound **2a** mp 248–250° (decomp.) (TLC, mmp).

Costusoside J (20 mg) was refluxed with MeOH (10 ml) for 10 hr and crystallized from MeOH to give costusoside I, mp 220–224° (decomp.) (TLC, mmp).

CrO_3 *oxidation of acetate* **2b**. The acetate (**2b**, 450 mg) in HOAc (30 ml) was refluxed for 3 hr and evaporated to dryness *in vacuo*. The residue was crystallized from Et_2O–hexane to give a white powder (430 mg). This product (430 mg) was dissolved in 80% HOAc (35 ml) and CrO_3 (410 mg) in 80% HOAc (2 ml) was added dropwise stirring constantly and cooling below 20°. The mixture was stirred for another 4 hr and the excess of the reagent decomposed with MeOH. The product thus obtained was extracted with Et_2O and washed with H_2O and the solvent removed. The residue (390 mg) was hydrolysed by refluxing with K_2CO_3 in *iso*-PrOH (5%, 30 ml) for 1.5 hr. The hydrolysate was diluted with H_2O (150 ml) and extracted with aq. *n*-BuOH. The *n*-BuOH layer was washed with H_2O, the solvent removed under red. pres. and the residue crystallized from MeOH to give **5** as colourless crystals mp 230–232° (decomp.). UV λ_{max}^{MeOH} nm: 238; IR ν_{max}^{KBr} cm^{-1}: 3700, 3200, 1660, 1150–1000, 760.

Compound **5** was hydrolysed with 1N H_2SO_4 in 50% Me_2CO [15] and the products were identified as 3β-hydroxy-pregna-5,16-dien-20-one (**7**), mp 203–205° and traces of pregna-3,5,16-trien-20-one (**8**), D-glucose and L-rhamnose by comparison with H_2O authentic samples.

The H_2O layer left after extraction with *n*-BuOH was brought to pH 6 with HOAc and once again extracted with *n*-BuOH satd with H_2O. The *n*-BuOH was removed under vacuum, and the residue obtained repeatedly crystallized from MeOH to give γ-methyl-δ-hydroxy pentanoic acid glucoside (**6**) as colourless needles (35 mg, mp 263–267° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 3400, 1700 (broad), 1040, 760. This material (10 mg) was incubated with almond emulsin for 6 days to give D-glucose (PC). Acetylation with Ac_2O–pyridine, followed by methylation with CH_2N_2 and purification through chromatography gave an oil which was found to be identical with **3** by TLC (System F).

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