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STEROIDAL SAPONINS OF *COSTUS LACERUS*

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ABSTRACT

Acid hydrolysis of the rhizomes of Costus lacerus *Gagn. yielded diosgenin in 1.7 percent. The 95% ethanolic extract of the rhizomes yielded β-sitosterol-β-D-glucoside, prosapogenin A, dioscin and gracillin. Partial hydrolysis of gracillin gave the new saponin, 3-O-[β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl] diosgenin.*

INTRODUCTION

Diosgenin is an important intermediate for the synthesis of steroidal drugs. The rhizomes of *Costus speciosus* (Zingiberaceae) have been found to be a rich source of diosgenin.[1,2] Continued investigations of the constituents of the roots has led to the identification of 5α-stigmast-9 (11)-en-3β-ol,[3] and several aliphatic compounds.[4,5] A number of saponins were also isolated and identified.[6,7] The seeds of *C. speciosus* were also found to be an additional source of diosgenin.[8] Studies of the *C. speciosus* seeds have yielded ten steroidal saponins.[9]

We now report the chemical investigation of the rhizomes of *Costus lacerus* Gagn., a plant common throughout the highlands of Thailand at about 1000 m above sea level. From the rhizomes of *C. lacerus,* diosgenin **1**, β -sitosterol-β-D-glucoside **3**, prosapogenin A **4**, dioscin **5** and gracillin **6** were isolated and identified.

RESULTS AND DISCUSSION

Acid hydrolysis of the dry rhizomes of *C. lacerus* yielded diosgenin **1** (1.7%), indicating that the species is a possible commercial source of diosgenin.

Repeated chromatography of the *n*-butanolic extract, obtained from the 95% ethanolic extract of the rhizomes, over silica gel with $CHCl_3$/MeOH/H_2O gave four crystalline saponins **3-6** : 3-*O*-[β-D-glucopyranosy]-β-sitosterol **3**,[7] 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] diosgenin (prosapogenin A)**4**,[10,11] 3-*O*-{[α-L-rhamnopyranosyl-(1→4)-[β-L-rhamnopyranosyl-(1→2)-]-β-D-glucopyranosyl} diosgenin (dioscin) **5**[10,12] and 3-*O*-{[β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl} diosgenin (gracillin) **6**.[6,12]

Acid hydrolysis of compounds **4-6** gave diosgenin **1** as the only sapogenin. The sugar residues of all the saponins **4-6** were identified as β-D-glucose and α-L-rhamnose. β-Sitosterol and β-D-glucose were identified upon acid hydrolysis of saponin **3**.

Partial hydrolysis (1% H_2SO_4 in 50% EtOH) of **5** gave **4** and 3-*O*-[β-D-glucopyranosyl] diosgenin (trillin) **7**; saponin **4** gave compound **7**. Compound **6** also yielded **7**, and, in addition, a new saponin, 3-*O*-[β-D-glucopyranosyl (1→3)]-[β-D-glucopyranosyl] diosgenin **8**. This result is in contrast with that obtained by Tschesche and Pandey who found that partial hydrolysis of gracillin **5** with 0.15*N* HCl in dioxane gave prosapogenin A **4** and **7**.[6]

Compounds **1-8** were characterized by their spectral data (IR, ^1H NMR) and chemical studies. Saponins **5** and **6** were also identified on the basis of their ^{13}C NMR spectra and MS (molecular ions). ^1H NMR spectral data of saponin acetates **11-15** are shown in Table 1; ^{13}C NMR data of saponins **4-6** are in agreement with the data previously reported.[13,14]

EXPERIMENTAL

Unless otherwise stated, analyses were carried out by Scientific and Technological Research Equipment Center, Chulalongkorn University, Bangkok, Thailand. Melting points were measured with a micromelting point apparatus. Infrared spectra were obtained with a Jasco A-302 spectrophotometer. ^1H NMR spectra of $CDCl_3$ solutions were recorded with a Bruker WM-400 MHz spectrometer. The ^{13}C NMR spectra were obtained at 100.62 MHz. CI mass spectra were determined with a Finnigan MAT TSQ46 instrument (desorption probe), methane being used as reagent gas. E.I. spectra were measured on an AEI-MS902 instrument. Optical rotations were measured for chloroform solutions with a Perkin-Elmer 241 spectropolarimeter. Plates for thin-layer chromatography (TLC) or preparative thin-layer chromatography (prep. TLC) were prepared from Merck silica gel PF254 and were activated by drying at 100° for 2 h. Silica gel 70-230 mesh (Merck) or Lichroprep RP-18 25-40 μm (Merck) was used for column chromatography. Acetate derivatives were prepared by the standard procedure (acetic anhydride/pyridine at room temperature). A voucher specimen (Chermsiriwattana 3297) of the plant material has been lodged at the Botany Section, Botany and Weed Science Division, Department of Agriculture, Bangkhen, Bangkok, Thailand.

Extraction and Isolation of Diosgenin

Dry and powdered rhizomes of *C. lacerus* (500 g) were mixed with a solution of sodium acetate (20 mg) in water (1 l). The mixture was allowed to stand for 24 h and hydrolyzed with 5% HCl (2 l) for about 14 h. The hydrolysate mass was filtered and repeatedly washed with water till free from acid. The residue was dried and extracted with hexane (2 l) in a Soxhlet apparatus for 4 h. Concentration of the hexane extract gave crude diosgenin **1** as a slightly yellow solid (8.2 g, 1.7%). The solid was crystallized from 95%

EtOH to give colorless needles, m.p. 213-215° (lit.[6] 208-209°) (Found : C, 76.7; H, 10.3. Calc. for $C_{27}H_{42}O_3$. ½ H_2O : C, 76.6; H, 10.2%). $[\alpha]_D^{25}$ - 126.8° (*c*, 0.07). MS *m/z* (rel. int.) : 414 (M^+, 9%), 342 (12), 300 (27), 282 (54), 271 (22), 267 (15), 139 (100).

The acetate **2** crystallized from CH_2Cl_2-MeOH as colorless needles, m.p. 203-205° (lit.[5] 198°) (Found: C, 76.3; H, 9.8. Calc. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7%). $[\alpha]_D^{25}$ - 176.3° (*c*, 0.04). IR and ^1H NMR spectra of **1** and the acetate **2** are in good correspondence with their structures.

Extraction and Isolation of Steroidal Saponins

Dry and powdered roots of *C. lacerus* (1.42 kg) were extracted with 95% EtOH in a Soxhlet extractor. The ethanolic extract was concentrated to give a dark brown residue which was partitioned between water-*n*-BuOH (1:1). The *n*-butanolic layer was evaporated to give the crude saponin fraction as a brown residue (116.5 g). A portion (40.0 g) of the crude saponin was separated on a column of silica gel (1.5 kg) which was gradiently eluted with $CHCl_3$-MeOH-H_2O to give saponins **3-6** as slightly yellow solids (0.23, 0.96, 1.73 and 2.13 g, respectively).

3-O-[β-D-Glucopyranosyl]-β-sitosterol **3**

Compound **3** was purified by column chromatography using silica gel and $CHCl_3$-MeOH-H_2O (20:3:1, lower phase) as the eluent to give a colorless solid which was crystallized from MeOH-H_2O as colorless granules, m.p. 295° (lit.[7] 282-284°) (Found: C, 72.9; H, 10.5. Calc. For $C_{35}H_{60}O_6$: C, 72.9; H, 10.5%). $[\alpha]_D^{25}$ -46.7° (*c*, 0.23, DMF) (lit.[7] -45°). ν_{max}nujol cm^{-1}: 3400 (br), 1360, 1070, 1050, 1020.

The acetate **9** separated from MeOH as colourless needles, m.p. 170-171.5° (Found: C, 69.2; H, 9.3. Calc. for $C_{43}H_{68}O_{10}$: C, 69.3; H, 9.2%). $[\alpha]_D^{25}$ -29.2° (*c*, 0.22). ν_{max} $CHCl_3$ cm^{-1} : 2950, 1750, 1220, 1030. ^1H NMR δ : 0.68, s, CH_3; 0.81, d, *J* 8.0 Hz, CH_3; 0.84, d. *J* 8.0 Hz, CH_3; 0.92, d, *J* 7.0 Hz, CH_3; 0.99, s, CH_3; 2.00, 2.02, 2.05, 2.08, all s, 4 × $OCOCH_3$; 4.59, d, *J* 8.0 Hz, anomeric H; 5.36, m, olefinic H.

Hydrolysis of Compound **3**

A solution of the glycoside **3** (22 mg) in 5% HCl (3 ml) and dioxane (0.5 ml) was refluxed for 14 h. The reaction mixture was extracted with chloroform and the extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated to give the crude product (10 mg) which was purified by column chromatography using EtOAc-hexane (1:9) as the eluent to give β-sitosterol **10**, which crystallized from hexane as colorless plates, m.p. 139-140° (undepressed on admixture with an authentic sample). ν_{max} $CHCl_3$ cm^{-1} : 3600, 2950, 1380, 1230, 1040. ^1H NMR δ : 0.68, s, CH_3; 0.83, d, *J* 8.0 Hz, CH_3; 0.84, d, *J* 8.0 Hz, CH_3; 0.85, t, *J* 8.0 Hz, CH_3; 0.93, d, *J* 7.0 Hz, CH_3; 1.01, s, CH_3; 0.7-2.32, m, CH and CH_2; 3.53, m. C*H*OH; 5.35, m, olefinic H. MS *m/z* (rel. int.): 414 (M^+, 37%), 399 (15), 396 (26),

383 (2), 381 (17), 329 (27), 303 (28), 273 (18), 255 (29), 231 (15), 213 (29), 178 (11), 163 (27), 161 (30), 105 (100).

The acidic aqueous layer was neutralized with Amberlite IRA-93 (OH-form) and filtered. The filtrate was evaporated to give a brown residue which was examined by TLC [silica gel, CH_2Cl_2-MeOH-H_2O (65:60:5, lower phase)]and identified as β-D-glucose.

3-O-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] diosgenin (prosapogenin A) **4**

Compound **4** was purified by chromatography on silica gel using $CHCl_3$-MeOH-H_2O (20:3:1 and 10:3:1) to give **4** as a colorless solid which was crystallized from MeOH-H_2O as a colorless granules, m.p. 257.5-258.5° (lit.[16] 244°, lit.[10] 230-245°, lit.[11] 225-235°) (Found: C, 63.1; H, 8.7. Calc. for $C_{39}H_{62}O_{12}$. H_2O : 63.2; H, 8.7%). $[\alpha]_D^{25}$ -112.8° (*c*, 0.21, DMF) (lit.[7] -97°). ν_{max} nujol cm^{-1} : 3400, 1375, 1045.

The crude product obtained on acetylation was purified by column chromatography using silica gel and CH_2Cl_2-MeOH (99:1) to give the acetate derivative **11**, colorless needles from MeOH, m.p. 207-209° (lit.[10] 207-209°, lit.[11] 198-200°) (Found: C, 63.1; H, 8.7. Calc. for $C_{39}H_{62}O_{12}$. H_2O : C, 63.2; H, 8.7%). $[\alpha]_D^{25}$ - 40.0° (*c*, 0.11). ν_{max} $CHCl_3$ cm^{-1} : 2950, 1740, 1360, 1220, 1040. ^1H NMR δ : 0.79, s, CH_3-18; 0.79, d, *J* 7.0 Hz, CH_3-27; 0.97, d, *J* 7.0 Hz, CH_3-21; 1.02, s, CH_3-19; 1.19, d, *J* 7.0 Hz, RhaCH$_3$; 2.00, 2.02, 2.03, 2.07, 2.08, 2.19, all s, 6×OCOCH$_3$; 3.37, t, *J* 11.0 Hz, H16; 3.47, m, CH_2-26; 4.58, d, *J* 8.0 Hz, GluH1; 4.97, d, *J* 1.5 Hz, RhaH1; 5.39, d, *J* 5.0 Hz, olefinic H.

Hydrolysis of Compound **4**

A mixture of compound **4** (2 mg), dioxane (5 drops) and 5% HCl (15 drops) was refluxed for 4 h. The reaction mixture was centrifuged to give a precipitate which was identified as diosgenin **1** by TLC comparison with an authentic specimen. The aqueous phase was neutralized with $NaHCO_3$ and was found to contain β-D-glucose and α-L-rhamnose by TLC comparison [silica gel, CH_2Cl_2-MeOH-H_2O (65:60:5, lower phase)] with the authentic β-D-glucose and α-L-rhamnose.

Partial Hydrolysis of Compound **4**

Compound **4** (1 mg) in 1% H_2SO_4 in 50% aqueous EtOH (1 ml) was refluxed for 2 h. The reaction mixture was extracted with *n*-BuOH. Evaporation of the *n*-butanolic extract gave a crude product which was identified as diosgenin **1** and trillin **7** by TLC comparison with the authentic samples **1** and **7**.

3-O-{[α-L-Rhamnopyranosyl-(1→4)]-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl}-diosgenin (dioscin) **5**

Compound **5** was purified on a column of silanised silica gel (70-230 mesh) using MeOH-H_2O (9:1) as the eluent to yield **5**, colorless needles from MeOH, m.p. 293-295° (lit.[16] 281°, lit.[10] 275-277°) (Found: C, 57.5; H, 8.2. Calc. for $C_{45}H_{72}O_{16}$. 4 H_2O : C, 57.4;

H, 8.8%). $[\alpha]_D^{25}$ - 120.7°(*c*, 0.37, DMF) (lit.[10] -115°). ν_{max} nujol cm^{-1} : 3250 (br). 1375, 1040. CIMS *m/z* (rel. int.) : 869 $[M + H]^+$ (37%), 723 [869-Rha + H]$^+$ (76), 577[723-Rha+H]$^+$ (26), 415[869-Rha-Glu+H]$^+$ (53), 397[415-H_2O]$^+$ (100).

The octaacetate **12**, obtained after chromatography of the crude product on a column of silica gel eluted with chloroform, crystallized from hexane as colorless rhombics, m.p. 151-154° (lit.[16]142-148°, lit.[10] 143-145°, lit[17] 145-147°) (Found: C, 60.2; H, 7.4. Calc. for $C_{61}H_{88}O_{24}$. H_2O : C, 59.9; H, 7.4%). $[\alpha]_D^{25}$-72° (*c*, 0.28) (lit[10] -46°). ν_{max} $CHCl_3$ cm^{-1} : 2950, 1740, 1365, 1210 (br), 1040 (br). ^1H NMR δ : 0.79, s, CH_3-18; 0.80, d, *J* 8.0 Hz, CH_3-27; 0.98, d, *J* 8.0 Hz, CH_3-21; 1.17, d, *J* 6.0 Hz, RhaCH$_3$; 1.19, d, *J* 6.0 Hz, RhaCH$_3$; 1.99, 2.02, 2.04, 2.09, 2.13, all s, 8×OCOCH$_3$; 3.37, t, *J* 11.0 Hz, H16; 3.45-3.50, m, CH_2-26; 4.56, d, 8.0 Hz, GluH1 4.79, d, *J* 1.5 Hz, RhaH1; 4.90, d,*J* 1.5 Hz, RhaH1 ; 5.40, d, *J* 5.0 Hz, olefinic H.

Hydrolysis of Compound 5

Saponin **5** (15 mg) in 5% HCl (2 ml) and dioxane (0.5 ml) was heated under reflux for 19 h. The precipitate formed was collected by centrifugation and identified as diosgenin **1** by TLC comparison with the authentic specimen. Examination of the aqueous hydrolysate by TLC [silica gel, CH_2Cl_2-MeOH-H_2O (65:60:5, lower phase)] revealed the presence of β-D-glucose and α-L-rhamnose.

Partial Hydrolysis of Compound **5**

A mixture of **5** (87 mg) in 1% H_2SO_4 and 50% aqueous EtOH (5 ml) was heated under reflux for 3 h. The reaction mixture was extracted with *n*-BuOH, and the *n*-butanolic extract was evaporated to give the crude product which was chromatographed on a column of silica gel using CH_2Cl_2-MeOH-H_2O (20:3:1 and 10:3:1) as the eluent to give diosgenin **1**(9 mg), trillin **7** (15 mg), prosapogenin A **4** (18 mg) and compound **5** (8 mg, recovered).

Crystallization of trillin **7** from MeOH-H_2O gave colorless needles, m.p. 282-285° (lit.[15] 196°, lit.[10] 250-255°) (Found : C, 66.6; H, 8.9. Calc. for $C_{33}H_{52}O_8$. H_2O : C, 66.6; H, 9.2%) $[\alpha]_D^{25}$-98.8° (*c*, 0.17). The acetate **13** crystallized from MeOH as colorless granules, m.p. 213-214° (Found: C, 65.9; H. 8.2. Calc. for $C_{41}H_{60}O_{12}$: C, 66.1; H, 8.1%). $[\alpha]_D^{25}$ -85.7°(*c*, 0.15). ν_{max} $CHCl_3$ cm^{-1}: 2950, 1750, 1370, 1220, 1040, 980, 920, 900. ^1H NMR δ: 0.78, s, CH_3-18; 0.79, d, *J* 7.0 Hz, CH_3-27; 0.97, d, *J* 7.0 Hz, CH_3-21; 2.00, 2.02, 2.05, 2.07, all s, 4×OCOCH$_3$; 3.37, t, *J* 11.0 Hz, H16; 3.47, m, CH_2-26; 3.68, ddd, *J* 9.5, 5.0, 2.5 Hz, GluH5; 4.11, dd, *J* 12.0, 2.5 Hz, GluH6a; 4.25, dd, *J* 12.0, 5.0 Hz, GluH6b; 4.59, d, *J* 8.0 Hz, GluH1; 4.95, dd, *J* 8.0, 9.5 Hz, GluH2; 5.07, t, *J* 9.5 Hz, GluH4; 5.20, t, *J* 9.5 Hz, GluH3; 5.36, d, *J* 5.0 Hz, olefinic H.

Prosapogenin A **4** was crystallized from MeOH-H_2O as colourless granules, m.p. 255-257°. $[\alpha]_D^{25}$ -122.7° (c, 0.17). Its IR spectrum and TLC behaviour were identical with those of the natural compound **4**. The acetate had m.p. 207-208°, undepressed on admixture with the derivative **11**. IR spectra and TLC behaviour of both samples **11** were identical.

Hydrolysis of Compound **7**

A mixture of compound **7** (1.2 mg), dioxane (10 drops) and 5% HCl (20 drops) was refluxed for 14 h. The precipitate obtained on centrifuging the reaction mixture was identified as diosgenin by TLC comparison with authentic diosgenin **1**. The aqueous hydrolysate was shown by TLC to contain β-D-glucose.

3-O-{[β-D-Glucopyranosyl-(1→3)]-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl}-diosgenin (gracillin) **6**

Compound **6** was crystallized from MeOH as colorless needles, m.p. 290-292° (lit.[6] 298-302°) (Found : C, 58.7; H, 8.3. Calc. for $C_{45}H_{72}O_{17}$. H_2O: C, 58.7; H, 8.3%). $[\alpha]_D^{25}$ -97.4°(*c*, 0.34, DMF) (lit.[6] -86.2°). ν_{max} Nujol cm^{-1} : 3400 (br), 1370, 1040. ^1H NMR δ:0.77, s, CH_3; 0.78, d, *J* 8.0 Hz, $CHCH_3$; 0.96, d, *J* 8.0 Hz, $CHCH_3$; 1.00, s, CH_3; 0.90-2.50, m, CH_2 and CH; 3.28-5.17, m, CH_2, CH and OH. CIMS *m/z* (rel. int.) : 739 [885-Rha + H]$^+$ (21%); 723 [885-Glu + H]$^+$ (53); 577 [723-Rha + H]$^+$ (32); 415 [885-Rha-Glu-Glu + H]$^+$ (38); 397 [415-H_2O]$^+$ (100). The acetate **14** crystallized from MeOH as colourless needles, m.p. 216-217° (lit.[6] 208°) (Found : C, 59.1; H, 7.0. Calc. for $C_{63}H_{90}O_{26}$. H_2O : C, 59.1; H, 7.3%). $[\alpha]_D^{25}$ -88.5° (*c*, 0.29) (lit.[6] -45°). ν_{max} $CHCl_3$ cm^{-1} : 2948, 1740, 1360, 1230, 1040. ^1H NMR δ : 0.79, s, CH_3-18; 0.80, d, *J* 6.5 Hz, CH_3-27; 0.97, d, *J* 7.0 Hz, CH_3-21; 1.03; s,CH_3-19; 1.18, d, *J* 6.0 Hz, RhaCH$_3$; 1.95, 1.99, 2.01, 2.03, 2.04, 2.06, 2.08, 2.22, 9×$OCOCH_3$; 3.37, t, *J* 10.5 Hz, H16; 3.48, m, CH_2-26; 4.46, d, *J* 8.0 Hz, GluH1; 4.72, d, *J* 8.0 Hz, GluH1; 5.25, d, *J* 1.5 Hz, RhaH1; 5.39, m, olefinic H.

Hydrolysis of Compound **6**

Glycoside **6** (15 mg) in 5% HCl (2 ml) and dioxane (0.5 ml) was heated under reflux for 24 h. The reaction mixture was worked up by the method described for hydrolysis of **5**, to give diosgenin **1**; the aqueous layer was found to contain β-D-glucose and α-L-rhamnose. Compound **1**, β -D-glucose and α-L-rhamnose were identified by TLC comparison with the authentic samples.

Partial Hydrolysis of Compound **6**

A mixture of compound **6** (100 mg) in 1% H_2SO_4 in 50% aqueous EtOH (7 ml) was refluxed for 7 h. The *n*-butanolic extract obtained was evaporated to give the crude product which was chromatographed on a column of silica gel using CH_2Cl_2-MeOH-H_2O (20:3:1) and (10:3:1) as the eluent to give compounds **1** (11 mg), **7** (22 mg), **8** (19 mg) and **6** (14 mg, recovered).

Compound **1** was crystallized from acetone as colorless needles, m.p. 211-214°, undepressed on admixture with authentic diosgenin. IR spectrum and TLC behaviour of **1** were identical with those of the authentic diosgenin.

Compound 7 was crystallized from MeOH-H_2O as colorless granules, m.p. 276-279° $[\alpha]_D^{25}$ -102.1° (*c*, 0.14). IR spectrum and TLC behaviour were identical with those of 7 obtained from the hydrolysis of **5**.

Compound **8** was crystallized from MeOH-H_2O as colourless granules, m.p. 274-278° $[\alpha]_D^{25}$ -79.5° (*c*, 0.17, DMF). ν_{max}KBr cm^{-1}: 3400, 2950, 1450, 1375, 1150, 1080, 1040, 1030, 1010, 900. The crude product obtained on acetylation was chromatographed on a silica gel column; elution with CH_2Cl_2-MeOH (99:1) gave **15**, which crystallized from MeOH as colorless plates, m.p. 254-255° (Found: C, 61.7; H, 7.4. Calc. for $C_{53}H_{76}O_{20}$: C, 61.6; H, 7.4%). $[\alpha]_D^{25}$ -86.0° (*c*, 0.04). ν_{max} $CHCl_3$ cm^{-1} 2950, 1750, 1365, 1225, 1040, 990, 925, 900. ^{1}H NMR δ : 0.78, s, CH_3-18; 0.79, d, *J* 7.0 Hz, CH_3-27; 0.97, d, *J* 7.0 Hz, CH_3-21; 0.99, s, CH_3-19; 1.89, 2.008, 2.01, 2.00, 2.07, 2.08, 2.13, all s, 7×$OCOCH_3$; 3.37, t, *J* 11.0 Hz, H16; 3.46, m, CH_2-26; 4.44, d, *J* 8.5 Hz, GluH1; 4.59,d, *J* 8.0 Hz, GluH1; 5.34, d, *J* 5.0 Hz, olefinic H.

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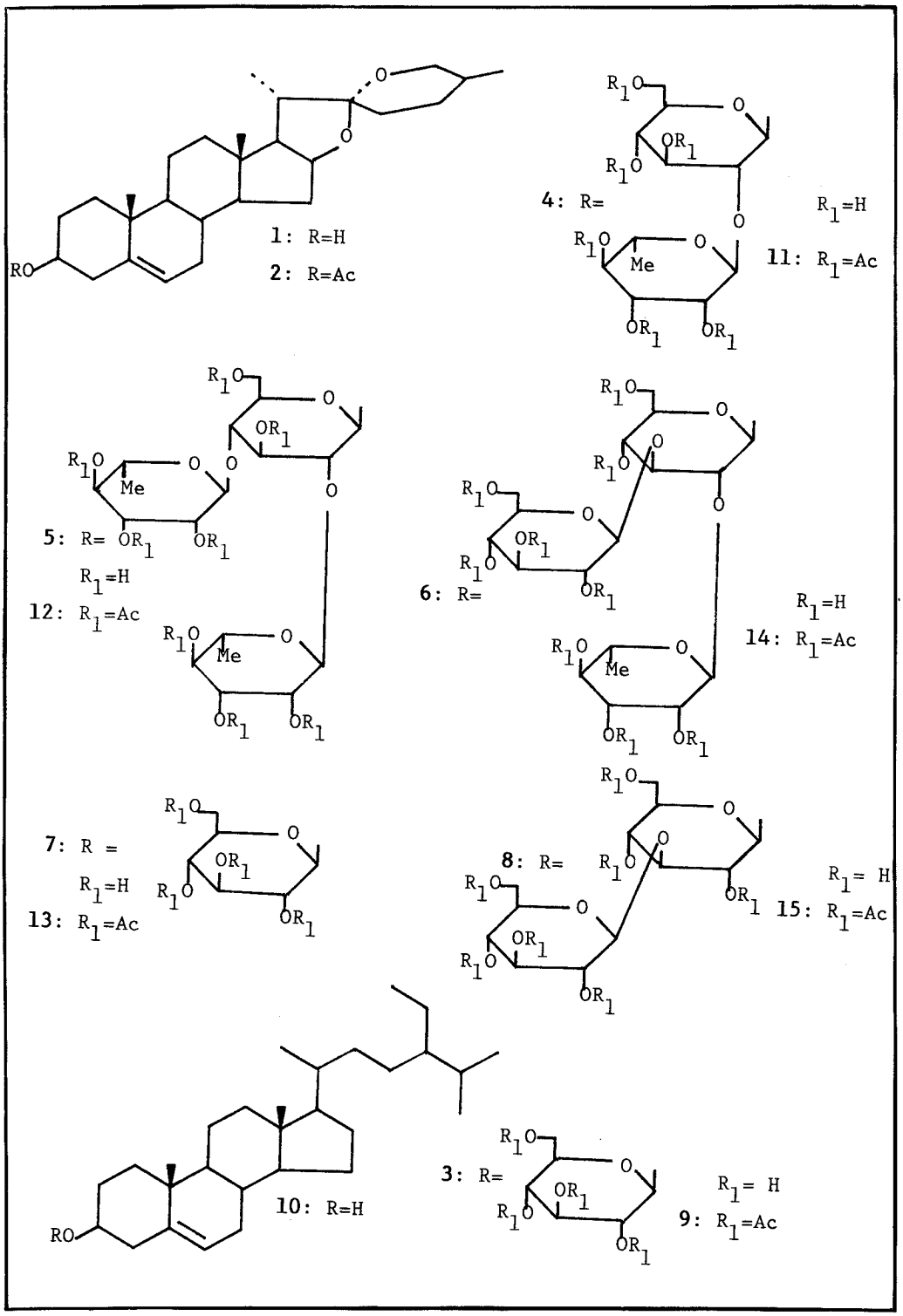
บทคัดย่อ

ไฮโดรไลซีส ของหัวเอื้องหมายนา *Costus lacerus* วงศ์ Zingiberaceae ด้วยกรดให้ diosgenin 1.7 เปอร์เซนต์ จากการตรวจสอบส่วนสกัดแอลกอฮอล์ของหัวเอื้องหมายนา พบสารประกอบ 4 ชนิดคือ β-sitosterol-β-D-glucoside, prosapogenin A, dioscin และ gracillin ไฮโดรไลซีสแบบไม่สมบูรณ์ของ gracillin ด้วยกรด ให้ซาโปนินตัวใหม่คือ 3-*O*-[β-D-glucopyranosyl-(1 $\rightarrow$3)]-[β-D-glucopyranosyl]diosgenin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2: R=Ac
4: R=
R1=H
11: R1=Ac
5: R=
R1=H
12: R1=Ac
6: R=
R1=H
14: R1=Ac
7: R =
R1=H
13: R1=Ac
8: R=
R1= H
15: R1=Ac
10: R=H
3: R=
R1= H
9: R1=Ac