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A SPIROSTANOL GLYCOSIDE FROM *AGAVE CANTALA*

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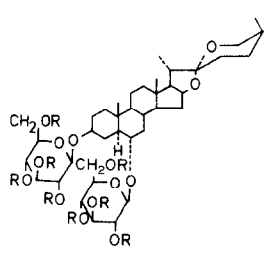
Key Word Index—*Agave cantala*; Agavaceae; saponins; spirostanol glycoside; 3-*O*-[β-D-glucopyranosyl]-6-*O*-[β-D-glucopyranosyl]-(25*R*)-5α-22α-*O*-spirostan-3β, 6α-diol.

Abstract—A new steroidal saponin has been isolated from the ethanolic extract of the roots of *Agave cantala* and shown to be 3-*O*-[β-D-glucopyranosyl]-6*O*-[β-D-glucopyranosyl]-(25*R*)-5α-22α-*O*-spirostan-3β, 6α-diol.

Agave cantala Roxb (Agavaceae) grows [1] wild in Himachal Pradesh (India) and this species exhibits diuretic, antiscorbic, antisyphilitic and anticancer properties [2]. A number of steroidal sapogenins have been reported [3–6] from this plant. This paper describes the isolation of a new spirostanol glycoside (**1**) having two sugar chains (which is rare) and gives details of its structure elucidation.

Repeated CC of the crude saponin mixture of the leaves afforded the saponin (**1**), $C_{39}H_{64}O_{14}$, mp 245–246°, which gave colour reactions of steroids and showed characteristic spiroketal absorptions in its IR spectrum. On hydrolysis it afforded D-glucose and the aglycone, which was identified (IR, ^{1}H NMR, MS and the physical constants of its acetyl derivatives) as (25*R*)-5α, 22α-*O*-spirostan-3β, 6α-diol (chlorogenin).

To determine the structure of the glycone moiety, **1** was permethylated by Hakomori's method [7] to give a permethylate (**2**), $C_{47}H_{80}O_{14}$, mp 177–179°. The mass spectrum of **2** showed peaks at *m/z* 868 $[M]^+$, 633 $[M-(\text{tetra-}O\text{-methyl D-glucose})+H]^+$, 398 $[M-2\ \text{tetra-}O\text{-methyl-D-glucose}-H]^+$, 219, 187[terminal D-glucose moiety]$^+$, indicating that both of the hydroxyl groups of chlorogenin were glycosidated with one molecule each of D-glucose. This was confirmed by the hydrolysis of **2** to afford the aglycone (chlorogenin) and 2, 3, 4, 6-tetra-*O*-methyl-D-glucose. The identity of 2, 3, 4, 6-tetra-*O*-methyl-D-glucose was further confirmed by the mass spectrum of methyl pyranoside, obtained by the methanolysis of **2**, which showed fragmention peaks in accordance with the expected pattern [8]. Enzymatic hydrolysis of **1** with β-glucosidase yielded D-glucose only, indicating that D-glucose molecules were linked to the aglycone through β-linkages. The above assignment of the glycosidic linkages in **1** was also supported by the application of Klyne's rule [9].



1 R=H
2 R=Me

Thus **1** was characterized as 3-*O*-[β-D-glucopyranosyl]-6-*O*-[β-D-glucopyranosyl]-(25*R*)-5α-22α-*O*-spirostan-3β, 6α-diol.

EXPERIMENTAL

Mps are uncorr. Spots on TLC were developed by 7% H_2SO_4 and on PC by aniline hydrogen phthalate. Well-dried and coarsely powered leaves (2.5 kg) of the plant were exhaustively extracted with EtOAc. The extract was removed and the solvent free leaves were further extracted with EtOH until the extracts became colourless. The solvent-free EtOH extract was purified as usual for the isolation of the saponins. The residue thus obtained was repeatedly chromatographed over Si gel ($CHCl_3$–MeOH–H_2O, 65 : 30 : 10) to give TLC homogeneous saponin **1** (2.5 g).

Saponin **1**. Crystallized from MeOH as colourless needles, mp 245–246°, $[\alpha]_D^{20}$ − 78.0° ($CHCl_3$–MeOH, *c* = 0.5). IR ν_{max}^{KBr} cm^{-1}: 965, 930, 907, 870 (intensity 907 > 930, 25*R*-spiroketal). (Found C, 64.95; H, 8.72. $C_{39}H_{64}O_{14}$ requires: C, 65.82; H, 8.46%.)

Hydrolysis of **1**. Compound **1** (350 mg) was hydrolysed by refluxing with 7% H_2SO_4 for 5 hr, cooled and filtered to give an aglycone, crystallized from $CHCl_3$ as colourless needles, mp 275–276°, $[\alpha]_D^{20}$ − 45° ($CHCl_3$, *c* = 0.7). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 980, 950, 915, 895 (intensity 895 > 915, 25*R*-spiroketal). EIMS (probe) 70 eV, *m/z* (rel. int.): 432 $[M]^+$ (9.0), 417 $[M-CH_3]^+$ (1.9), 414 $[M-H_2O]^+$ (0.4), 363 (10.0), 360 (26.0), 318 (16.2), 303 (11.5), 300 (8.8), 289 (26.3), 271 (10.3), 253 (3.3), 139 (100), 115 (20). ^{1}H NMR (90 MHz, $CDCl_3$–CF_3COOH, TMS as internal reference): δ 0.70 (3 H, *s*, H-18), 0.80 (3 H, *d*, H-27), 0.90 (3 H, *d*, H-21), 0.97 (3 H, *s*, H-19), 4.50 (1 H, *q*, H-16). (Found: C, 74.25; H, 10.10. Calc. for $C_{27}H_{44}O_4$ C, 74.89; H, 10.88%.)

Chlorogenin-diacetate. Prepared as usual, mp 150–152°, $[\alpha]_D^{20}$ − 38° ($CHCl_3$, *c* = 1.0) (lit. [10]): mp 151°).

The aq. hydrolysate was neutralized (Ag_2CO_3), filtered and

concd. It showed D-glucose (PC; *n*-BuOH–AcOH–H_2O, 4 : 1 : 5; R_f 0.16).

Permethylate of saponin **1**. Compound **1** (1 g) was permethylated to give a crude product (**2**) which was purified by CC (C_6H_6–EtOAc, 1:1) and crystallized from MeOH as colourless silky needles, mp 177–179°, $[\alpha]_D^{20} - 75.0°$ ($CHCl_3$, $c = 1.0$); IR ν_{max}^{KBr} cm^{-1}: no OH; EIMS (probe) 70 eV, *m/z* (rel. int.): 868 $[M]^+$ (6), 633 (12), 414 (7), 400 (10), 399 (15), 398 (75), 253 (6), 219 (20), 187 (100). (Found: C, 64.80; H, 9.15. $C_{47}H_{80}O_{14}$ requires: C, 64.97; H, 9.21%).

Hydrolysis of **2**. Compound **2** (150 mg) was hydrolysed with 7% H_2SO_4 as usual, the ppt was filtered, crystallized with $CHCl_3$ and identified as chlorogenin. The aq. hydrolysate was neutralized (Ag_2CO_3), filtered and concentrated to provide 2, 3, 4, 6-tetra-*O*-methyl-D-glucose (PC; *n*-BuOH–EtOH–H_2O, 5 : 1 : 4; R_G 1.0, authentic sample run in parallel).

Methanolysis of **2**. Compound **2** (150 mg) in NHCl–MeOH (25 ml) was refluxed for 4 hr, neutralized (Ag_2CO_3), filtered and the filtrate was concentrated and purified by CC (C_6H_6–Me_2CO, 10 : 1) to give methyl-2, 3, 4, 6-tetra-*O*-methyl-D-glucopyranoside (10 mg): EIMS (probe) 70 eV, *m/z* (rel. int.): 219 (0.04), 205 (0.08), 187 (0.35), 175 (3.1), 149 (7.0), 145 (1.8), 131 (2.5), 127 (9.0), 104 (45), 88 (100), 75 (25), 73 (10), 71 (10), 45 (20).

Enzymatic hydrolysis of **1**. Compound **1** (50 mg) in H_2O (10 ml) was incubated with β-glucosidase (Sigma) at 37° for 7 hr. The hydrolysate was filtered. The ppt. was identified as chlorogenin. The aq. phase contained D-glucose only (PC, as above).

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DEOXYRADICININ, A NOVEL PHYTOTOXIN FROM *ALTERNARIA HELIANTHI*

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Key Word Index—*Alternaria helianthi*; Dematiaceae; *Helianthus annuus*; Compositae; sunflower; phytotoxin; deoxyradicinin.

Abstract—A novel major metabolite which possesses phytotoxic and antifungal properties has been isolated from culture filtrates of *Alternaria helianthi* and its structure elucidated as deoxyradicinin.

INTRODUCTION

Alternaria helianthi (Hansf.) Tubaki and Nishihara (Dematiaceae) is a phytopathogenic fungus causing seedling blight and leaf spot of sunflower [*Helianthus annuus* L. (Compositae)] [1], a crop plant of considerable economic importance [2]. In a previous paper the isolation and identification of the phytotoxic pyranopyrone radicinin from *A. chrysanthemi*, a pathogen of *Leucanthemum maximum* (Ram.) DC. (shasta daisy), was described [3]. *A. helianthi* and *A. chrysanthemi* are reported to be morphologically indistinguishable [4] and are pathologically similar in that both species