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A STEROIDAL GLYCOSIDE FROM *CLERODENDRON INERME*

ATTA-UR-REHMAN,* SAEEDAN BEGUM,†‡ SUMAYYA SAIED,† M. IQBAL CHOUDHARY* and FARZANA AKHTAR*

† Department of Chemistry, University of Karachi, Karachi-75270, Pakistan; * H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

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Abstract—A steroidal glycoside, 3-*O*-β-D-galactopyranosyl-(24β)-ethylcholesta-5,22,25-trien (**1**), was isolated from *Clerodendron inerme*. Clerosterol and glutinol were also isolated from this plant. © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Clerodendron inerme (L.) Gaertn. is a medicinal plant found growing along the coastal belts of Sri Lanka [1]. It is used in the indigenous system of medicine for the treatment of scrofulous and venereal infections, skin diseases, beri-beri and also as an antidote for poisoning from fish, crabs and toadstools [2, 3]. Previous phytochemical investigations on this plant have resulted in the isolation of clerodane diterpenes [4–7], diterpene quinones [8], flavonoids [9–11], triterpenes [8], sterols [8, 9] and neolignans [12]. We report here the isolation and identification of a steroidal glycoside together with clerosterol and a triterpene, glutinol.

RESULTS AND DISCUSSION

Compound **1** was isolated from the aerial parts and was purified by TLC. It gave a purple-blue colouration with the sugar reagent. Acid hydrolysis of **1** liberated a sugar moiety which was identified as β-D-galactose by TLC and spectroscopic comparison with authentic sugar samples.

The negative-ion FAB mass spectrum revealed a pseudomolecular ion peak at m/z 571 $[M-H]^-$. The elemental analysis of **1** corresponded to the molecular formula $C_{35}H_{54}O_6$. The EI mass spectrum showed a peak at m/z 392 in the highest mass region, which corresponded to the loss of the galactose moiety. The other peaks at m/z 213, 229, 255, 271, 281 and 299 indicated the presence of sterol skeleton on the aglycone with three degrees of unsaturation, one in the nucleus and two in the side chain [13–17].

The ^{1}H NMR and ^{13}C NMR chemical shifts in the intact glycoside (see Experimental) revealed that the aglycone of **1** was (24β)-ethylcholesta-5,22,25-trien-3β-ol by comparison with reported data [18–21]. In the ^{13}C NMR spectrum the downfield chemical shift of C-3 of the aglycone (δ 77.0) indicated the linkage of the sugar moiety at this carbon. The signal for the anomeric carbon appeared at δ 100.8. The comparison of the chemical shifts of carbons of the sugar moiety with the reported data further confirmed the presence of galactose.

On the basis of the above evidence the structure of **1** was established as 3-*O*-β-D-galactopyranosyl-(24β)-ethylcholesta-5,22,25-trien. We also isolated clerosterol [15, 19, 22] and glutinol [23, 24] as a new source from this plant.

EXPERIMENTAL

Plant material. Aerial parts of *C. inerme* were collected from the campus of the University of Karachi in August 1991.

Extraction and isolation. The plant was air-dried in the shade and soaked in EtOH for 1 week. The ethanolic extract was evapd under red. pres. to obtain a gummy residue which was then partitioned with *n*-hexane, EtOAC and *n*-BuOH. The hexane extract was hydrolysed with KOH in EtOH. The unsaponified hexane extract (25 g) after evapn under red. pres. was subjected to CC on silica gel grade 60 (70–230 mesh) eluted with mixts of *n*-hexane, $CHCl_3$ and MeOH. Compound **1** was obtained as colourless crystals from the fr. eluted with $CHCl_3$–MeOH, 10:0.5 and after further purification by prep. TLC on silica gel G $CHCl_3$–MeOH (8:2). The fraction eluted with hexane–$CHCl_3$ (7:3) yielded glutinol. Clerosterol was

‡ Author to whom correspondence should be addressed.

obtained in crystalline form on elution with *n*-hexane–$CHCl_3$ (4:6). It was recrystallized from MeOH. Glutinol and clerosterol were identified by comparison of their spectroscopic data with the published data [14, 15, 22–25].

3-O-*β*-D-*galactopyranosyl*-(24*β*)-*ethylcholesta*-5,22,25-*trien* (**1**). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH groups), 1650 (trisubstituted double bond), 1160–1000 (C—O stretch), 965 (trans disubstituted double bond), 880, 635 (>C=CH_2 group). Negative FAB-MS *m/z*: 571 $[M-H]^-$. ^1H NMR (DMSO-d_6, 400 MHz): δ 4.20 (1H, *d*, *J* = 7.8 Hz, H-1′), 5.30 (1H, distorted *t*, H-6), 5.22 (1H, *dd*, *J* = 7.6, 15.2 Hz, H-22), 5.16 (1H, *dd*, *J* = 7.3, 5.2 Hz, H-23), 4.66 (2H, *s*, H-26), 3.38 (overlapped signals for OH groups), 1.59, 0.94, 0.65 (9H, *s*, H-27, H-19, H-18), 0.96 (3H, *d*, *J* = 6.7 Hz, H-21), 0.77 (3H, *t*, *J* = 7.3 Hz, H-29). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 36.9 (C-1), 31.4 (C-2), 77.0 (C-3), 38.4 (C-4), 140.5 (C-5), 121.2 (C-6), 29.3 (C-7), 31.5 (C-8), 49.7 (C-9), 36.3 (C-10), 21.1 (C-11), 38.4 (C-12), 41.8 (C-13), 56.3 (C-14), 23.9 (C-15), 28.4 (C-16), 55.3 (C-17), 11.9 (C-18), 19.1 (C-19), 40.4 (C-20), 19.9 (C-21), 136.8 (C-22), 129.6 (C-23), 51.3 (C-24), 147.7 (C-25), 110.0 (C-26), 20.7 (C-27), 25.3 (C-28), 12.0 (C-29), 100.8 (C-1′), 73.5 (C-2′), 76.7 (C-3′), 70.1 (C-4′), 76.8 (C-5′), 61.1 (C-6′).

Acid hydrolysis of **1**. Steroidal glycoside **1** (18.9 mg) was hydrolysed with $CHCl_3$–MeOH–HCl (37%) (42:42:16) under reflux for 1 hr. The resulting mixt. was concd under red. pres., after H_2O had been added, the residue was extracted with Et_2O. The Et_2O extract was washed with H_2O, dried with dry Na_2SO_4 and evapd under red. pres. The aqueous layer was neutralized with Ag_2CO_3 and evapd to dryness under red. pres. The presence of D-galactose was shown by comparison with standard sugars on silica gel TLC developed in a system of *n*-BuOH–EtOAc–*iso*-PrOH–HOAc–H_2O (35:100:60:35:30). The spots were detected by spraying with aniline phthalate reagent.

Acetylation of aglycone of **1**. The aglycone of **1** (36.3 mg) obtained after acid hydrolysis was subjected to acetylation by refluxing it with 3 ml pyridine and 7.5 ml Ac_2O for 3 hr. The steryl acetate, purified by prep. TLC on silica gel, was identical with the acetate of (24*β*)-ethylcholesta-5,22,25-trien-3*β*-ol, also isolated as a free sterol from the unsaponifiable matter of *C. inerme* by comparison with its mass, IR (KBr), ^1H NMR and ^{13}C NMR spectral data.

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