Phytochemistry, 1980, Vol. 19, pp. 1875–1876. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0801-1875 $02.00/0

PRIDENTIGENIN E, A TRITERPENOID SAPOGENIN FROM *PRIMULA DENTICULATA*

VIQAR UDDIN AHMAD*, QAZI NAJMUS SAQIB† and K. USMANGHANI‡

H. E. J. Postgraduate Institute of Chemistry, University of Karachi, Karachi-32, Pakistan

(*Received* 29 *November* 1979)

Key Word Index—*Primula denticulata*; Primulaceae; triterpenoid sapogenin; pridentigenin E; dihydrocyclamiretin D.

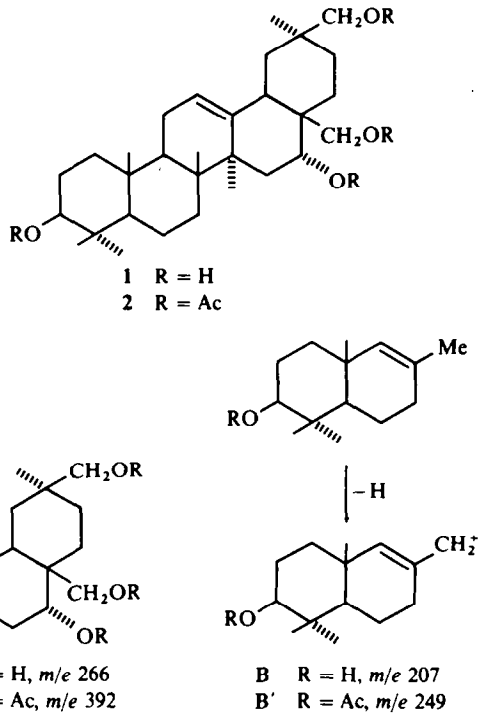
Abstract—The triterpenoid sapogenin pridentigenin E, isolated from *Primula denticulata*, has been identified as dihydrocyclamiretin D.

We have recently reported the isolation of five sapogenins from *Primula denticulata* Sm., which were provisionally named as pridentigenin A,B,C,D and E [1]. A structure for pridentigenin B was also proposed. In the present communication we report the structure of pridentigenin E.

Pridentigenin E was isolated by alumina column chromatography of the crude sapogenin mixture. After purification by PLC and recrystallization from methanol, pridentigenin E (**1**) had mp 268–270°. The UV spectrum of this sapogenin showed no maxima above 220 nm, excluding the presence of conjugated double bonds. In the IR spectrum (KBr), a broad band at 3340 cm^{-1} (OH), a peak at 1635 cm^{-1} (C = C stretching) and a peak at 825 cm^{-1} characteristic of a double bonds at the C-12 (13) position in a pentacyclic triterpenoid [2] were visible. In the ^1H NMR spectrum (C_5D_5N) four singlets due to six tertiary methyl groups were seen at δ 1.08 (2 × Me), 1.25 (1 × Me), 1.40 (1 × Me) and 1.84 (2 × Me). Two broad singlets at 3.71 and 3.97 were assigned to —CH_2OH groups. A broad signal at 4.64 was typical of the C-12 proton.

The MS of pridentigenin E showed a weak molecular ion peak at *m/e* 474. Other important peaks were at *m/e* 456 (M − H_2O), 443 (M − CH_2OH), 425 (M − CH_2OH − H_2O), 266 (fragment A), 248 (A − H_2O), 235 A − CH_2OH), 207 (fragment B), 189 (B − H_2O).

On acetylation with acetic anhydride and pyridine, pridentigenin E yielded a tetraacetate (**2**) which could not be obtained in a crystalline form. The IR spectrum ($CHCl_3$) of the chromatographically pure tetraacetate showed no absorption due to a hydroxyl group. There was a band at 1725 cm^{-1} due to the acetate groups. In the 100 MHz ^1H NMR spectrum ($CDCl_3$) this derivative showed the presence of six tertiary methyl groups with singlets at δ 0.81 (2 × Me), 1.90 (1 × Me), 0.93 (2 × Me), 1.24 (1 × Me).



1 R = H
2 R = Ac

A R = H, *m/e* 266
A′ R = Ac, *m/e* 392

B R = H, *m/e* 207
B′ R = Ac, *m/e* 249

The four singlets at 2.03, 2.04, 2.06 and 2.07 corresponded to four acetyl groups. An AB-quartet centred at 3.87 (A H, J_{AB} = 7.2 Hz) was apparently due to two magnetically equivalent —CH_2OAc groups. The signals at 4.48 (1 H, *t*, C-3 H), 5.10 (1 H, C-22 H) and 5.35 (1 H, C-16 H) were in conformity with the assignments in other triterpenoids of similar structure.

In the MS of pridentigenin E tetraacetate, the molecular peak was not visible. The highest peak at *m/e* 582 was due to the elimination of a molecule of acetic acid from the molecular ion (M − 60). Other important peaks were at *m/e* 522 (M − 2 × HOAc), a weak peak due to fragment A′ at *m/e* 392 but stronger ones at *m/e* 332 (A′ − HOAc), 272 (A′ − 2 × HOAc), 212 (A′ − 3 × HOAc). The base

* Author to whom correspondence should be addressed.

† Present address: Department of Pharmacy, Gomal University, Dera Ismail Khan (NWFP), Pakistan.

‡ Present address: Department of Pharmacognosy, University of Karachi, Karachi-32, Pakistan.

peak at *m/e* 199 corresponded to the elimination of two molecules of acetic acid and one —CH_2OAc fragment from A′.

On the basis of the above spectra, it is suggested that pridentigenin E is identical to dihydrocyclamiretin D. This compound was first prepared by Barton *et al.* [3] and later by Tschesche [4] through the reduction of cyclamiretin by borohydride. This is the first report of its occurrence in nature. Since an authentic sample of dihydrocyclamiretin could not be obtained, we prepared it through the borohydride reduction of our sample of cyclamiretin which we obtained through hydrolysis of pridentigenin B. The identity of pridentigenin E with the dihydrocyclamiretin so obtained was proved by mmp, TLC and superimposable IR spectra of the two samples.

EXPERIMENTAL

The residue obtained on evapn of the ethanolic extract of *Primula denticulata* (whole plant, 2.5 kg), was fractionated between *n*-BuOH and H_2O. The crude saponin mixture (200 g) obtained on evapn of the BuOH layer was dissolved in MeOH, pptd with Et_2O and then hydrolysed by refluxing with 2 N HCl in dil. EtOH for 4 hr. The mixture of sapogenins (20 g) obtained was chromatographed on a neutral alumina column. The final fractions eluted with $CHCl_3$–MeOH (4:1) and pure MeOH afforded pridentigenin E along with more polar and less polar constituents. Pridentigenin E was purified by PLC on Si gel plates using $CHCl_3$–MeOH (4:1) as developing solvent. The bands were made visible by spraying with H_2O and the main band of pridentigenin E was scraped off and eluted with MeOH. Pridentigenin E, obtained on crystallization of the residue from MeOH, had mp 268–270.

Acetylation of pridentigenin E. Pridentigenin E (50 g) was dissolved in Ac_2O (1 ml) and Py (0.5 ml) and kept for 24 hr at room temp. The reaction mixture was then poured onto ice and extracted exhaustively with Et_2O. The etheral layer was washed successively with H_2O, 5% HOAc, H_2O, 5% $NaHCO_3$ and finally again with H_2O. After drying (Na_2SO_4) and evapn, a gummy residue of pridentigenin E was obtained which was chromatographically pure but failed to crystallize.

Borohydride reduction of cyclamiretin D. Cyclamiretin D obtained on acid hydrolysis of pridentigenin B (1) was dissolved in EtOH and a soln of $NaBH_4$ (10 mg) in H_2O was added. The reaction mixture was kept overnight, the excess $NaBH_4$ was decomposed by the addition of dil HOAc, evapd, taken up in H_2O and filtered. The ppt. was crystallized from MeOH, the dihydrocyclameritin D so obtained had mp 268–270°. It was identified as pridentigenin E through mmp and superimposable IR spectra. For all spectral data see text.

REFERENCES

1. Ahmad, V. U., Najmus-Saqib, Q. and Usmanghani, K. (1980) in press.
2. Cole, A. R. N. and Thornton, D. W. (1957) *J. Chem. Soc.* 1332.
3. Barton, D. R. H., Hameed, A. and McGhie, J. F. (1962) *J. Chem. Soc.* 5176.
4. Tschesche, R., Striegler, H. and Fehlhaber, H. W. (1966) *Liebigs Ann. Chem.* **691**, 165.