*Phytochemistry*, 1980, Vol. 19, pp. 980–981. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0501-0980 $02.00/0

# α-L-RHAMNOPYRANOSYL-3β-HYDROXY-LUP-20(29)-EN-28-OIC ACID FROM THE STEM OF *DILLENIA PENTAGYNA*

KAMALA P. TIWARI, SAVITRI D. SRIVASTAVA and SANTOSH K. SRIVASTAVA*

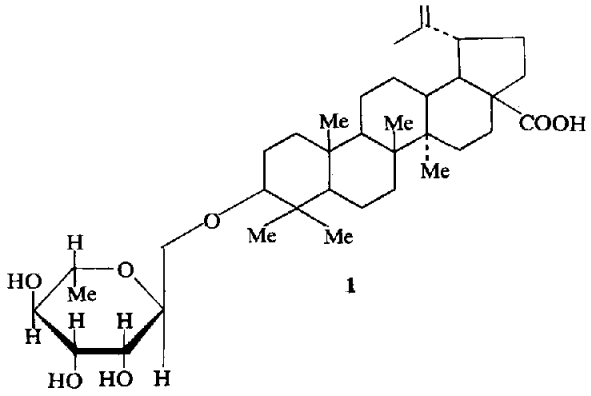
Chemistry Department, Allahabad University, Allahabad-211002, India

(*Received* 14 *May* 1979)

**Key Word Index**—*Dillenia pentagyna*; Dilleniaceae; α-L-rhamnopyranosyl-3β-hydroxy-lup-20(29)-en-28-oic acid; a new saponin.

## INTRODUCTION

*Dillenia pentagyna* [1, 2] is a plant of high medicinal value employed in our indigenous system of medicines. No work on this plant appears in the literature and therefore we have instigated an investigation of its chemical constituents. We report in this paper the isolation and identification of a new saponin named α-L-rhamnopyranosyl-3β-hydroxy-lup-20(29)-en-28-oic acid (**1**) on the basis of spectral and chemical evidence.




**1**

## RESULTS AND DISCUSSION

A new triterpene glycoside, mp 290° (d) showed characteristic properties of a saponin. It gave a copious foam when shaken with water, haemolysed red blood cells and was toxic to fish. Hydrolysis (7%, $H_2SO_4$) of the glycoside yielded a colourless aglycone (sapogenin) and a sugar which was identified as L-rhamnose by co-PC and osazone formation.

The aglycone, mp 316–17°, $[\alpha]_D^{25}+8°$ (in $C_6H_5N$), $C_{30}H_{48}O_3$, $M^+$ 456, gave a positive Liebermann–Burchard test [3] and a yellow colour with TNM [4] specific for triterpenoids. Its MS showed fragments at *m/e* 441 (M−Me), 438 (M−$H_2O$), 423 (M−Me−$H_2O$), 411 (M−COOH), 207 (base peak) and 189 as in the case of lupane series [5]. It formed a monoacetate ($Ac_2O$–Py), mp 290–91°, $M^+$ 498, IR acetyl absorption at 1740 $cm^{-1}$ and disappearance of the hydroxyl absorption (3450 $cm^{-1}$). Its $^1$HNMR displayed a singlet at δ2.9 for one acetoxyl group which showed the presence of a hydroxyl group in the sapogenin. The IR spectrum showed a vinylidene group (3080, 1639 and 885 $cm^{-1}$) and the $^1$H NMR spectrum exhibited the presence of five methyls, a vinylic methyl (δ1.75), one methine proton (3.21), and a doublet at 4.53 ($J$ = 6Hz) expected for a vinylidene group. The aglycone also gave a positive test for unsaturation. The IR, and $^1$H NMR spectra and MS fragmentation pattern of the sapogenin clearly established that the hydroxyl group was located in ring A or B and that the double bond, was at position C-20 : C-29 in the lupane series. The $^1$H NMR spectrum (δ5.4) indicated the presence of a secondary hydroxyl in the sapogenin. The secondary nature of the hydroxyl was further confirmed by the formation of a monoketone by $CrO_3$ oxidation [6], mp 166–67°. It gave a positive Zimmermann test [7] indicating that the hydroxyl group was at C-3 in the aglycone. The aglycone formed a methyl ester with $CH_2N_2$ (mp 221–22°, $M^+$ 470, NMR signal at δ3.75 (*s*)) as shown by the appearance of the carbonyl ester absorption at 1720 $cm^{-1}$ and disappearance of the carbonyl absorption (1680 $cm^{-1}$) of the −COOH group in the IR spectrum, thus establishing the presence of a −COOH group. The methyl ester of the aglycone on saponification with 10% methanolic KOH for 8 hr at the reflux temperature resulted in partial hydrolysis (85% of the ester was recovered unchanged). However, its saponification with diethyleneglycollic KOH regenerated the original aglycone, mp 316–17°. The above facts clearly indicated that the −COOH group was highly hindered and it was attached to C-17 [8, 9]. The methyl ester on reduction with $LiAlH_4$ in a mixture of tetrahydrofuran–ether (1 : 4) furnished a diol, $C_{30}H_{50}O_2$, mp 250–52° which was identified as betulin [10], by mp, mmp, co-IR and

* Correspondence address: Chemistry Department, Saugar University, Sagar (M.P.)-470-003, India.

co-chromatography with an authentic sample. The $-CH_2OH$ at C-17 in betulin resulted from the reduction of the $-COOMe$ group of the methyl ester and accordingly, the parent triterpenic acid contained $-COOH$ at C-17.

Thus on the basis of the foregoing results the aglycone was established as 3-hydroxy-lup-20(29)-en-28-oic acid which was confirmed by co-chromatography with an authentic sample.

Periodate oxidation [11] (consumed 2 mol of periodate and 1 mol HCOOH liberated) and enzymatic hydrolysis of the glycoside indicated the presence of the sugar L-rhamnose as the monosaccharide $\alpha$-glycosidically linked in the pyranose form to the aglycone molecule.

On this basis structure **1** has been assigned to the saponin.

## EXPERIMENTAL

*Isolation and purification.* The air-dried and powdered stem (2 kg) of *Dillenia pentagyna* (collected from the Botany Department, University of Allahabad, Allahabad and identified by the Botanical Survey of India, Allahabad Circle) was extracted with EtOH under reflux. The extract (2.5 l.) was filtered concd to 100 ml and poured into excess $H_2O$. The insoluble material was washed thoroughly with $H_2O$ and extracted with $C_6H_6$. The $C_6H_6$ extract on concn deposited a brown solid mass. This material was then extracted with $CHCl_3$. The $CHCl_3$-soluble fraction after concn deposited the glycoside (yield 2 g). It was purified on an alumina column (MeOH–$CHCl_3$). The glycoside was repeatedly crystallized as a colourless amorphous powder from EtOH (yield 1.92 g). It was found to be homogeneous on TLC ($R_f$ 0.48, $CHCl_3$–MeOH, 8:2, sprayed with $SbCl_3$ in $CHCl_3$), $[\alpha]_D^{25}+28°$ (in $C_6H_5N$), mp 290°(d), $M^+$ 530. (Found: C, 71.89; H, 9.29. $C_{36}H_{58}O_7$ requires: C, 71.76; H, 9.63%).

*Acidic hydrolysis.* The glycoside (1 g) was hydrolysed with 80 ml (7%, ethanolic $H_2SO_4$) for 5 hr under reflux. The reaction product was poured into excess of $H_2O$ (300 ml). On cooling a solid ppt. was obtained which was washed with $H_2O$ and crystallized as colourless needles from MeOH. The aq. hydrolysate after neutralization ($BaCO_3$) was identified as L-rhamnose (co-PC and osazone). The aglycone, mp 316–17°, $M^+$ 456, was found to be homogeneous on TLC ($R_f$ 0.44 in $CHCl_3$–MeOH, 9:1, spray $SbCl_3$ in $CHCl_3$), $[\alpha]_D^{25}+8°$ (in $C_6H_5N$), UV $\lambda_{max}^{EtOH}$ nm: 204; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450 (OH), 2933, 2850 (d, C–H str. in cyclopentane nucleus) [12] 3070, 1639, 885 (vinylidene), 1680 (carboxyl), 1620, 1460, 1390, 1380, 1362, 1260, 1120, 1042, 1015, 948, 892, 885. (Found: C, 78.91; H, 10.51. $C_{30}H_{48}O_3$ requires: C, 78.91; H, 10.52%). MS *m/e*: 456 ($M^+$), 441 ($M^+-Me$), 438 ($M^+-H_2O$), 423 [$M^+-(Me+H_2O)$], 411 ($M^+-COOH$), 248, 220, 219, 207, 189, 187. It formed a monoacetate ($Ac_2O$/Py) mp 290–91°, $M^+$ 498, $[\alpha]_D^{25}+8°$ (in $CHCl_3$) (Found: C, 77.09; H, 10.20. $C_{32}H_{50}O_4$ requires: C, 77.10; H, 10.04%) IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2940, 2855, 1740 (acetate carbonyl), 1704 (carboxy carbonyl), 1640, 1240, 885; monobenzoate, mp 342°(d); $M^+$ 560 (Found: C, 79.26; H, 9.28. $C_{37}H_{52}O_4$ requires: C, 79.28; H, 9.28%) and a methyl ester ($CH_2N_2$), mp 221–22°, $[\alpha]_D^{25}+8°$ (in $CHCl_3$), $M^+$ 470 (Found: C, 78.89; H, 10.62. $C_{31}H_{50}O_3$ requires: C, 79.14; H, 10.64%), UV $\lambda_{max}^{EtOH}$ nm: 203. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3560 (OH), 1720 (ester carbonyl), 1640, 1460, 1360, 885. MS *m/e*: 470 ($M^+$), 455 ($M^+-Me$), 452 ($M^+-H_2O$), 437 [$M^+-(Me+H_2O)$], 411 ($M^+-COOMe$), 262, 233, 220, 207, 189 (base peak), 187. $^1H$ NMR ($CDCl_3$) 60 MHz, TMS int. standard): $\delta$0.75, 0.77, 0.90, 1.00, 1.15, 1.75, 3.75 (*s*, 3H as COOMe) and 4.6–4.8. The methyl ester of the aglycone (500 mg) was reduced with $LiAlH_4$ (500 mg) [9, 13] in THF (20 ml) and $Et_2O$ (50 ml) under reflux for 3 hr. The product obtained was identified as betulin (by co-TLC and IR), mp 250–52°, $M^+$ 442. (Found; C, 81.39; H, 11.30. $C_{30}H_{50}O_2$ requires; C, 81.44; H, 11.31%). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3510(OH), 2965, 1645, 1462, 1380, 1110, 1085, 1020, 985, 975, 885. The saponin (25 mg) was dissolved in EtOH (15 ml) and emulsin solution (15 ml) (prepared from almonds) and hydrolysed by the usual process. The saponin was recovered unchanged thus proving the $\alpha$-glycosidic linkage.

*Acknowledgement*—The authors are grateful to the Director, CIBA Research Centre, Bombay, for providing the microanalysis and spectra and also SCST, UP, Lucknow (India), for financial assistance to one of us (S.D.S.).

## REFERENCES

1. (1952) *The Wealth of India*, p. 65. CSIR, New Delhi.
2. Kirtikar, K. R. and Basu, B. D. (1965) *Indian Medicinal Plants*, Vol. 3, p. 54. Lalit Mohan Basu, Allahabad, India.
3. Liebermann, C. (1885) *Ber. Dtsch. Chem. Ges.* **18,** 1804.
4. Ruzicka, L. and Liebig, S. (1929) *Ann. Chem.* **25,** 471.
5. Djerrasi, C., Budzikiewicz, H. and Wilson, J. M. (1963) *J. Am. Chem. Soc.* **85,** 3688.
6. Poos, G. I., Arth, G. E., Beyler, R. E. and Sarett, L. H. (1953) *J. Am. Chem. Soc.* **75,** 427.
7. Barton, D. H. R. and Mayo, P. de (1954) *J. Chem. Soc.* 887.
8. Shamma, M., Glick, R. E. and Mumma, R. O. (1962) *J. Org. Chem.* **27,** 4512.
9. Bhattacharjee, S. R. and Chatterjee, A. (1962) *J. Indian Chem. Soc.* **39,** 276.
10. Heilbron, I. and Bunburry, H. M. (1953) *Dictionary of Organic Compounds*, Vol. 1, p. 287. Eyre & Spottiswoode, London.
11. Hirst, E. L. and Jones, J. K. N. (1949) *J. Chem. Soc.* 928.
12. Rao, C. N. R. (1963) *Chemical Application of Infra-red Spectroscopy*, p. 144.
13. Djerrasi, C. (1955) *J. Chem. Soc.* **77,** 1825.