Phytochemistry, 1972, Vol. 11, pp. 3061 to 3063. Pergamon Press. Printed in England.

3-*EPI*-BETULINIC ACID, A NEW TRITERPENOID FROM *PICRAMNIA PENTANDRA*

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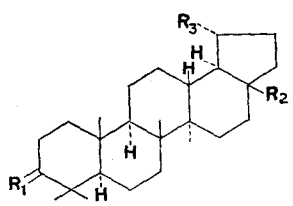
(*Received* 28 *March* 1972. *Accepted* 1 *May* 1972)

Key Word Index—*Picramnia pentandra*; Simaroubaceae; 3-*epi*-betulinic acid; triterpenoid.

Abstract—Extraction of the bark of *Picramnia pentandra* Sw. gave as the main component a new triterpenoid, whose structure was established as 3-*epi*-betulinic acid.

BECAUSE of current interest in the group of degraded triterpenes known as quassinoids,[1] constituents of many representatives of the family Simaroubaceae have been investigated, but to our knowledge there is no information on the relatively large genus *Pentandra*. We now report isolation of a new triterpenoid, 3-*epi*-betulinic acid, from the very bitter bark of *Picramnia pentandra* Sw., (bitter bush) the only representative of this genus found in the U.S. This is used as a fever remedy in the West Indies. Investigation of the wood gave only small amounts of amorphous material and nothing which could be identified as a quassinoid.

The hexane extract of the bark contained as a main component a triterpenoid (I), $C_{30}H_{48}O_3$, which according to the IR spectrum incorporated a hydroxyl group (3435 cm^{-1}), a carboxyl group (3400–3000 and 1705 cm^{-1}) and a terminal methylene group (3070, 1642 and 890 cm^{-1}). The NMR spectrum displayed signals due to six tertiary methyl groups, one of which was vinylic (1·7 ppm) and, as shown by double irradiation experiments, long-range coupled to the two vinylic protons of the terminal methylene group (4·60 and 4·77 ppm). Hydrogenation of I resulted in the formation of a dihydro derivative II and caused the replacement of the three vinylic signals in the NMR spectrum by two methyl doublets, thus establishing the presence of a terminal isopropenyl group in I.



(I) R_1 = H, OH(α); R_2 = COOH; R_3 = isopropenyl
(II) R_1 = H, OH(α); R_2 = COOH; R_3 = isopropyl
(III) R_1 = H, OH(α); R_2 = COOMe; R_3 = isopropenyl
(IV) R_1 = H, OH(α); R_2 = CH_2OH; R_3 = isopropenyl
(V) R_1 = H, OAc(α); R_2 = COOH; R_3 = isopropenyl
(VI) R_1 = O; R_2 = COOMe; R_3 = isopropenyl

The MS of I was in accordance with these results and exhibited diagnostically important peaks at *m/e* 456 (M), 438 (M-18), 423 (M-33), 410 (M-46), 248 (*A*), 220 (*B*), 219 (*C*), 207 (*D*), 203 (*A*-45) and 189 (*D*-18) (Fig. 1). This fragmentation pattern strongly indicated that

[1] J. D. CONNOLLY, K. H. OVERTON and J. POLONSKY, in *Progress in Phytochemistry* (edited by L. REINHOLD and Y. LIWSHITZ), Vol. II, p. 385, Interscience, New York (1970).

the compound was of the lup-20 (29)-ene type and allowed allocation of the carboxyl group to C-17 and that of the hydroxyl group to rings *A/B*.[2]

The presence of the carboxyl group was also confirmed by chemical means. Methylation of I yielded a methyl ester III (M = 470, M-59, *D* = 262; IR band at 1710 cm^{-1}; NMR peak at 3·68 ppm), while reduction with $LiAlH_4$ in dioxane afforded a diol IV (M = 442, M-31, *A* = 234). The NMR spectrum of the latter exhibited the typical *AB* doublets of the carbinol group at 3·32 and 3·80 ppm (*J* = 12).

The hydroxyl group was obviously secondary, since the NMR spectrum of compound I contained a one proton signal at 3·39 ppm, appropriately shifted to 4·62 ppm in the spectrum of the acetate V. Oxidation and subsequent methylation of I yielded an oxo methyl ester VI, whose physical[3] and spectral[4] data agreed well with those published for methyl betulonate.

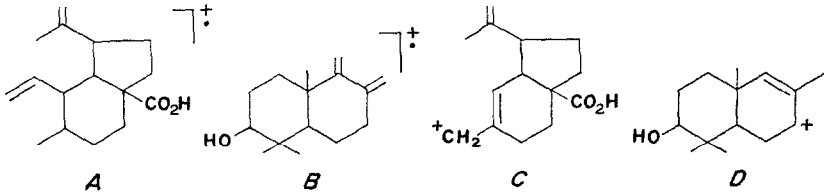


FIG. 1.

Having established the site of oxygenation as C-3, the axial orientation of the hydroxyl group follows from the small coupling constant ($J \sim 2$) of the methine proton in the NMR spectra of compounds I–V. Direct comparison of the methyl ester III and a synthetic sample of methyl 3-*epi*-betulinate eventually demonstrated that the compound obtained from *Picramnia pentandra* is 3-*epi*-betulinic acid. To our knowledge this acid is a new natural product and is an addition to the relatively few naturally-occurring pentacyclic triterpenoids which incorporate a 3α-hydroxyl group. Among these are 3-*epi*-lupeol which has previously been isolated from some *Bursera*[5] species, 11-oxo-3-*epi*-lupeol from *Fluorensia resinosa*[6] and most recently, 3-*epi*-betulin from *Canthium dicoccum* Gaertn.[7]

EXPERIMENTAL

M.ps. were determined in capillaries and are uncorrected. Rotations were run in $CHCl_3$ on a Jasco ORD/UV-5 recording spectrophotometer; IR spectra as KBr pellets; NMR spectra in $CDCl_3$, unless otherwise stated, on a Bruker 90-MHz instrument; MS on an MS-902 high resolution or a Nuclide 25 cm medium resolution mass spectrometer at 70 eV. Analyses were by Dr. F. Pascher, Bonn, Germany.

Isolation of 3-epi-*betulinic acid* (I). Ground bark, wt 0·9 kg of *Picramnia pentandra* Sw., collected by Mr. G. Avery on Big Pine Key, Florida (material collected by Mr. B. Cancel Collazo in the vicinity of Mayaguez, Puerto Rico, gave the same results) was extracted with hexane. The extract was chromatographed over silicic acid. The $CHCl_3$ eluate was fairly homogeneous. Recrystallization from acetone–hexane afforded 0·8 g of 3-*epi*-betulinic acid I, m.p. 279–283°; $[\alpha]_D^{25}$ −11° (*c*, 0·2); IR bands at 3435, 3400–3000, 3070, 1705, 1642 and 890 cm^{-1}, NMR signals ($CDCl_3$ containing a few drops of pyridine-d_5) at 0·80 (3H, s), 0·82 (3H, s), 0·95 (3H, s), 0·97 (9H, s), 1·71 (3H, broad s), 3·39 (1H, dd, $J \sim 2$), 4·60 (1H, br, $W_{1/2}$ = 5 Hz) and 4·77 ppm (1H, br, $W_{1/2}$ = 5 Hz); *m/e* 456 (M, 5), 438 (32), 423 (27), 410 (7), 248 (*A*, 12), 220 (*B*, 13), 219 (*C*, 13), 207

[2] H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).
[3] T. G. HALSALL and R. T. APLIN, in *Progress in the Chemistry or Organic Natural Products* (edited by L. ZECHMEISTER), Vol. 22, p. 153, Springer, Wien (1964).
[4] H. T. CHEUNG and D. G. WILLIAMSON, *Tetrahedron* **25**, 119 (1969).
[5] B. TURSCH and E. TURSCH, *Bull. Soc. Chim. Belg.* **70**, 585 (1961).
[6] H. ESTRADA, E. ESTRADA and L. MAYA, *Bol. Inst. Quim. Univ. Nacl. Auton. Mex.* **17**, 68 (1965).
[7] S. C. DAS, *Chem. & Ind.* 1331 (1971).

(*D*, 36), 203 (29) and 189 (100). (Found: C, 78·37; H, 10·39; O, 11·13. Calcd. for $C_{30}H_{48}O_3$: C, 78·89; H, 10·59; O, 10·51%.)

3-epi-*Dihydrobetulinic acid* (II). A solution of 0·15 g of I in 65 ml of MeOH was shaken with PtO_2 (80 mg) at 2·4 atm. hydrogen pressure for 8 hr. The solution was filtered, evaporated and chromatographed over silicic acid. Elution with $CHCl_3$ gave 3-*epi*-dihydrobetulinic acid II, which on recrystallization from hexane had mp.. 298–301°; $[\alpha]_D$ −46° (*c*, 0·1); IR bands at 3440, 3400–3000 and 1705 cm^{-1}, NMR signals at 0·74 (3H, d, $J = 6$), 0·81 (3H, s), 0·84 (3H, s), 0·84 (3H, d, $J = 6$), 0·92 (6H, s), 0·96 (3H, s) and 3·40 ppm (1H, dd, $J \sim 2$ Hz). *m/e*: 458 (M, 3), 440 (14), 425 (13), 412 (7), 221 (*C*,7), 220 (*B*, 3), 207 (*D*, 28), 203 (17) and 189 (100). (Found: C, 79·03; H, 10·57; O, 10·66. Calcd. for $C_{30}H_{50}O_3$, C, 78·55; H, 10·99; O, 10·46%.)

Methyl 3-epi-*betulinate* (III). On treatment with ethereal CH_2N_2 overnight, I gave methyl 3-*epi*-betulinate (III), m.p. 221–222°, undepressed on admixture with an authentic sample; $[\alpha]_D$ −16° (*c*, 0·25); reported −13°;[8] the IR, NMR and MS were identical with those of the authentic sample; IR bands at 3550, 3075, 1710, 1645 and 890 cm^{-1}; NMR signals at 0·86 (6H, s), 0·95 (6H, s), 1·01 (3H, s) 1·71 (3H, s br), 3·41 (1H, dd, $J \sim 2$), 3·68 (3H, s), 4·62 (1H, br) and 4·76 ppm (1H, br); *m/e*: 470 (M, 18), 452 (13), 437 (7), 411 (5), 410 (7), 262 (*A*, 34), 233 (*C*, 7), 220 (*B*, 22), 207 (*D*, 45), 203 (26) and 189 (100).

3-epi-*Betulin* (IV). A solution of 85 mg of I in 10 ml of dioxane was refluxed with excess $LiAlH_4$ for 4 hr. Dilution with aq. H_2SO_4 (20%), extraction with $CHCl_3$ and TLC over silica gel (hexane–EtOAc, 4:1) gave 3-*epi*-betulin (80 mg) which after recrystallization from hexane had m.p. 199–200°; $[\alpha]_D$ +7° (*c* 0·2); reported m.p. 215°; $[\alpha]_D$ +69°,[7] IR bands at 3400, 3080, 1645 and 895 cm^{-1}; NMR signals at 0·82 (6H, s), 0·92 (3H, s), 0·99 (3H, s), 1·02 (3H, s), 1·68 (3H, s br), 3·32 (1H, d, $J = 10·6$), 3·39 (1H, dd, $J \sim 2$), 3·80 (1H, d, $J = 10·6$), 4·60 (1H, br), 4·69 ppm (1H, br); *m/e*: 442 (M, 33), 424 (19), 411 (34), 234 (*A*,9), 220 (*B*, 10), 207 (*D*, 61), 203 (44), 189 (100). MW 442·3794, Calcd. for $C_{30}H_{50}O_2$ 442·3810.

3-epi-*Betulinic acid acetate* (V). A mixture of 0·1 g of I, 1 ml of Ac_2O and 0·4 ml of pyridine was allowed to stand at room temp. overnight. The product was recrystallized from acetone–hexane to yield 3-*epi*-betulinic acid acetate (V), m.p. 261–263°; $[\alpha]_D$ −21° (*c*, 0·3), IR bands at 3600–3100, 3070, 1740, 1690, 1640, 1248 and 892 cm^{-1}; NMR signals at 0·85 (9H, s), 0·94 (3H, s), 1·03 (3H, s), 1·69 (3H, s br), 2·06 (3H, s), 4·62 (1H, dd, $J \sim 2$), 4·62 (1H, br) and 4·75 ppm (1H, br); m/e: 498 (M, 17), 452 (3), 438 (95), 423 (28), 249 (*D*, 22), 248 (*A*, 39), 203 (33), 202 (28) and 189 (100). MW: 498 (low resolution MS). Calcd. for $C_{32}H_{50}O_4$: MW 498.

Methyl betulonate (VI). A solution of 0·1 g of I in 5 ml of acetone was allowed to stand with 0·5 ml of Jones' reagent at room temp. for 15 min. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. Chromatography over silica gel, methylation of the acid obtained with ethereal CH_2N_2 and recrystallization from acetonitrile yielded methyl betulonate, m.p. 161–163° $[\alpha]_D$ +29° (*c*, 0·1); reported m.p. 165°; $[\alpha]_D$ +31°.[3] The NMR spectrum agreed well with the data published by Cheung and Williamson[4] *m/e* 468 (M, 75), 409 (31), 408 (25), 393 (13), 262 (*A*, 81), 233 (*C*, 19), 218 (*B*, 31), 205 (*D*, 50), 203 (44) and 189 (100).

Acknowlegdements—This work was supported in part by grants from the U.S. Public Health Service (GM-05814 and CA-13121). I.W. wishes to thank the Swedish Tobacco Company for a stipend. We are greatly indebted to Dr. S. Huneck, Institut für Biochemie der Pflanzen, Halle, DDR, for authentic samples of triterpenoids.

[8] J. L. Allsop, A. R. H. Cole, D. E. White and R. L. S. Willix, *J. Chem. Soc.* 4868 (1956).