MARSUPSIN, A NEW BENZOFURANONE FROM PTEROCARPUS MARSUPIUM ROXB.

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Abstract - Chromatography of an ethyl acetate extract of defatted Pterocarpus Marsupium Roxb. (Leguminosae) heartwood over silica gel afforded marsupsin (1) which was characterized as a new 2-hydroxy-2-benzyl-3(2H)-benzofuranone by a consideration of physicochemical data and conversion to triacetylmarsupsin (3) and trimethylmarsupsin (tetramethylmaesopsin) (2).

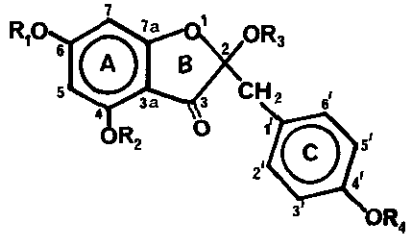
Pterocarpus Marsupium Roxb. (Leguminosae), also known as Indian Kino Tree or Bija Sar, is a large tree common to mixed deciduous forests of central and peninsular India[1]. Extracts of the leaves, flowers and gum of this tree have been used medicinally in the treatment of diarrhea, toothache, fever, and urinary tract and skin infections while extracts of the bark have long been regarded as useful in the therapy of diabetes.[1,2] Pterocarpus Marsupium heartwood (2.5 kg) was defatted with petroleum ether (60-80°C) and the defatted marc extracted with ethyl acetate. The ethyl acetate extract was concentrated and cooled to afford a precipitate which was filtered and the filtrate chromatographed over silica gel. Elution with benzene followed by benzene-ethyl acetate mixtures (19:1,9:1) afforded various fractions currently under study, while elution with benzene-ethyl acetate (3:1) afforded marsupsin (1) (150 mg) as white needles; mp 193-195°, $[\alpha]_D^{26}$-4° (c 0.5, MeOH); uv λ_{max}^{MeOH} nm (log ε) 212(4.25), 223(4.24) and 292(4.19), with a bathochromic shift on addition of 0.1N NaOH to 212(4.28), 227(sh)(4.14), 245(3.80), 291(sh)(3.70) and 328(4.41); ir ν_{max}^{KBr} cm^{-1} 3440, 3380, 1680 and 1600. The nmr spectrum (60 MHz, $CDCl_3$-CD_3OD(5:1), TMS, δ in ppm) indicated the presence of one benzylic methylene group at 3.10(2H,s), one aromatic methoxy group at 3.78(3H,s), two meta coupled aromatic protons at 5.87(1H,d,J=2Hz) and 5.91(1H,d,J=2Hz), and one A_2B_2 aromatic system at 6.60(2H,d,J=9Hz) and 7.05(2H,d,J=9Hz). The ms showed M^+ at m/z 302 (1%) for $C_{16}H_{14}O_6$ with other significant fragment ions at m/z 301(1), 284(2), 274(1), 195(38), 167(100), 152(9), 134(4), 124(9) and 107(41). A consideration of the spectral data suggested that marsupsin was a 2-benzyl-2,4,6,4'-tetraoxygenated-3(2H)-benzofuranone containing one phenolic hydroxy group and one aromatic methoxy group in ring A, one alcoholic hydroxy group

in ring B and one phenolic hydroxy group in ring C.[3-6] A consideration of the mass spectral fragment ions at m/z 195 (38%)(a), 167(100) (a-CO) and 107(41)(b) further supported this hypothesis.[7]

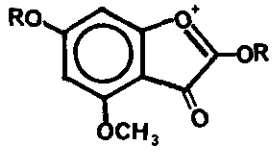
Treatment of marsupsin with ethereal diazomethane afforded trimethylmarsupsin(2) as an oil (which resisted all attempts at crystallization), uv λ_{max}^{MeOH} nm (log ε) 223(4.14), 292(4.09) and 360(sh) (2.63) with no bathochromic shift on addition of 0.1N NaOH; ir ν_{max}^{KBr} (neat) cm^{-1} 2940, 2850, 1710 and 1620. The nmr spectrum (60 MHz, $CDCl_3$, TMS, δ in ppm) indicated the presence of one benzylic methylene group at 3.11(2H,s), one aliphatic methoxy group at 3.27(3H,s), three aromatic methoxy groups at 3.72(3H,s) and 3.84(6H,s), two meta coupled aromatic protons at 5.90(1H,d,J=2Hz) and 6.05(1H,d,J=2Hz) and one A_2B_2 aromatic system at 6.72(2H,d,J=8Hz) and 7.17(2H,d,J=8Hz). The ms showed M^+ at m/z 344 (10%) with other significant fragment ions at 329(1), 313(1), 237(4), 223(100), 209(2), 195(3), 180(11), 167(5), 149(14) and 121(58). The lack of a bathochromic shift in alkali in the uv spectrum coupled with the addition of one aliphatic and two aromatic methoxy groups in the nmr spectrum and the addition of 42 amu (3x14 amu) in the M^+ of trimethylmarsupsin indicated that marsupsin contained two phenolic and one alcoholic hydroxy groups. (The alcoholic hydroxy group at C-2 of 2-hydroxy-2-benzyl-3(2H)-benzofuranones has been successfully methylated with diazomethane [4,9]). The significant fragment ions at m/z 223(c) and 121(d) further supported this hypothesis.[7] Finally, a direct comparsion (nmr) of tetramethylmaesopsin (2) and trimethylmarsupsin[8] showed them to be identical confirming the skeletal structure and positions of oxygenation of marsupsin. Furthermore, the fragment ions at m/z 107(b) and 121(d) in the ms of marsupsin and trimethylmarsupsin, respectively, confirmed the presence of a phenolic group at C-4' in ring C of marsupsin.[7] In addition, the presence of an aliphatic methoxy signal (δ3.27) in the nmr spectrum of trimethylmarsupsin further confirmed the presence of an aliphatic hydroxy group at C-2 in ring B of marsupsin. Remaining to be determined was the position of the second phenolic group which must be at either C-4 or C-6 in ring A.

Treatment of marsupsin with acetic anhydride and anhydrous sodium acetate afforded marsupsin triacetate (3) as an oil; uv, λ_{max}^{MeOH} nm (log ε) 218(3.95), 280(3.80) and 325(sh)(3.33) with a bathochromic shift on addition of 0.1N NaOH to 219(4.07), 241(3.96) and 319(4.08); ir ν_{max}^{KBr} (neat) cm^{-1} 2930, 2850, 1765 (phenolic acetate), 1735 (aliphatic acetate) 1720 and 1605. The nmr spectrum (600 MHz, $CDCl_3$, TMS, δ in ppm) indicated the presence of one aliphatic acetate group at 2.10(3H,s), two aromatic acetate groups at 2.27(3H,s) and 2.30(3H,s), one geminal coupled benzylic methylene group at 3.07(1H,d,J=14Hz) and 3.27(1H,d,J=14Hz), one aromatic methoxy group at 3.89(3H,s), two meta coupled aromatic protons at 6.23(1H,d,J=2Hz) and 6.39(1H,d,J=2Hz) and one A_2B_2 aromatic system at 6.97(2H,d,J=8.5Hz) and 7.27(2H,d,J=8.5Hz). The ms showed M^+ at m/z 428 (4%) for $C_{22}H_{20}O_9$ and other significant fragment ions at m/z 385 (1), 368(6), 326(15), 284(3), 237(8), 209(26), 195(33), 167(88), 149(9), 108(12) and 107(100). The ir spectral bands at 1765 cm^{-1} and 1735 cm^{-1} plus the aromatic and aliphatic acetate signals in the nmr spectrum and the increase of 126 amu (3x42 amu) in the M^+ of triacetylmarsupsin over marsupsin further confirmed the diphenolic, monoalcoholic character of marsupsin. There was a downfield shift of

both of the meta coupled aromatic protons on ring A, from δ5.87 and δ5.91 in marsupsin to δ6.23 and δ6.39 in triacetylmarsupsin which suggested that the phenolic hydroxy group should be located at C-6, since if it were located at C-4, a somewhat lesser downfield shift of the aromatic proton at C-7 would be expected. Most significant, however, was the absence of a bathochromic shift in the uv spectrum of marsupsin upon addition of $AlCl_3$ confirming the absence of a C-4 hydroxy group which is known to chelate in a 3-keto-4-hydroxy system.[10,11] Finally, the Gibbs test was also negative indicating a substituted para position to the phenol.[12] Hence, marsupsin may be characterized as 2,6-dihydroxy-2-(p-hydroxybenzyl)-4-methoxy-3(2H)-benzofuranone[13], a new member of the 2-hydroxy-2-benzyl-3(2H)-benzofuranone class. Other members of this class include alphitonin (2,4,6-trihydroxy-2-(3',4'-dihydroxybenzyl)-3(2H)-benzofuranone) from Alphitonia excelsa (Rhamnaceae)[14], maesopsin (2,4,6-trihydroxy-2-(4'-hydroxybenzyl)-3(2H)-benzofuranone) from Maesopsis eminii (Rhamnaceae)[15,16] and Phyllogeiton zeyheri (Leguminosae)[3], nigrescin (2,6,7-trihydroxy-2-(3',4'-dihydroxybenzyl)-3(2H)-benzofuranone) from Acacia nigrescens (Leguminosae),[4] 2,6-dihydroxy-2-(3'-hydroxy,4'-methoxy)-3(2H)-benzofuranone from Schinopsis balansae and S. lorentzii (Anacardiaceae)[17] and 2,6-dihydroxy-2-(3',4'-dihydroxybenzyl)-3(2H)-benzofuranone from Trachylobium verrucosum (Leguminosae)[7] and both Schinopsis balansae and S. lorentzii (Anacardiaceae)[17]. This is the first reported isolation of a 2-hydroxy-2-benzyl-3(2H)-benzofuranone from the genus Pterocarpus, although pterocarpans,[18] isoflavones,[18] deoxybenzoins,[18] 2-phenyl-chromans[18], stilbenes,[18] benzofurans,[18] chalcones[19] and dihydrochalcones[20] have been reported in various Pterocarpus species. The biosynthesis of marsupsin, like other 2-hydroxy-2-benzyl-3(2H)-benzofuranones, apparently proceeds through the cyclization of the corresponding intermediate α-hydroxychalcone keto-isomer to afford a five-membered heterocyclic ring with remarkable stability[21]. Finally, the phenolic and flavonoidal nature of marsupsin may explain the folkloric use of extracts of the plant in various infections.[22]



1 $R_1=R_3=R_4=H$, $R_2=CH_3$
2 $R_1=R_2=R_3=R_4=CH_3$
3 $R_1=R_3=R_4=COCH_3$, $R_2=CH_3$



a R=H= m/z 195
c $R=CH_3$= m/z 223

b R=H= m/z 107
d $R=CH_3$= m/z 121

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REFERENCES

1. S.K. Jain, 'Medicinal Plants', National Book Trust, New Delhi, 1968, pp. 116-118.

2. R.N. Chopra, I.C. Chopra, K.L. Handa and L.D. Kapur, 'Indigenous Drugs of India, 2nd Ed.,' U.N. Dhur and Sons Private Limited, Calcutta, 1958, p. 522.

3. F. du Volsteedt and D.G. Roux, Tetrahedron Lett., 1971, 1647.

4. T.G. Fourie, I.C. DuPreez and D.G. Roux, Phytochemistry, 1972, 11, 1763.

5. K. Nakanishi, 'Infrared Absorption Spectroscopy - Practical', Holden-Day, Inc., San Francisco, 1962, p. 42.

6. A.I. Scott, 'Interpretation of the Ultraviolet Spectra of Natural Products', Pergamon Press Ltd., Oxford, 1964, p. 146 and p. 155.

7. D. Ferreira, J.P. van der Merwe and D.G. Roux, J. Chem. Soc., Perkin I, 1974, 1492.

8. Kindly supplied by Professor D.G. Roux, Department of Chemistry, The University of the Orange Free State, Bloemfontein, Republic of South Africa.

9. H. Zollinger, 'Azo and Diazo Chemistry', Interscience Publishers Inc., New York, 1961, p. 69

10. T.A. Geissman, 'The Chemistry of Flavonoid Compounds', The MacMillan Company, New York, 1962, p. 141.

11. J.B. Harborne, T.J. Mabry and H. Mabry, 'The Flavonoids, Part 1', Academic Press, New York, 1975, p. 61.

12. F.E. King, T.J. King and L.C. Manning, J. Chem. Soc., 1957, 563.

13. Dr. Kurt L. Loening, Director of Chemical Nomenclature, Chemical Abstracts Service, Columbus, Ohio 43210 has kindly informed us that the preferred IUPAC name for marsupsin is 2,6-dihydroxy-2-(p-hydroxybenzyl)-4-methoxy-3(2H)-benzofuranone.

14. A.J. Birch, E. Ritchie and R.N. Speake, J. Chem. Soc., 1960, 3593.

15. N.F. Janes, F.E. King and J.W.W. Morgan, Chem. and Ind., 1961, 346.

16. N.F. Janes, F.E. King and J.W.W. Morgan, J. Chem. Soc., 1963, 1356.

17. H.G.C. King, T. White and R.B. Hughes, J. Chem Soc., 1961, 3234.

18. T.R. Seshadri, Phytochemistry, 1972, 11, 881.

19. R.G. Cooke and I.D. Rae, Aust. J. Chem., 1964, 17, 379.

20. B.C.B. Bezuidenhoudt, E.V. Brandt and D.G. Roux, J. Chem. Soc., Perkin I, 1981, 263.

21. D.G. Roux and D. Ferriera, Phytochemistry, 1974, 13, 2039.

22. J.B. Harborne, T.J. Mabry and H. Mabry, 'The Flavonoids, Part 2', Academic Press, New York, 1975, pp. 1032-1033.

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