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NAPHTHOQUINONES FROM *EUCLEA PSEUDEBENUS*

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Key Word Index—*Euclea pseudebenus*, Ebenaceae, naphthoquinones, diospyrin; euclein, isodiospyrin, 8′-hydroxydiospyrin, racemic 8′-hydroxyisodiospyrin.

Abstract—Diospyrin, isodiospyrin, 8′-hydroxydiospyrin, racemic 8′-hydroxyisodiospyrin and two other naphthoquinones have been isolated from *Euclea pseudebenus* Euclein is recognized to be identical to diospyrin

THE CHARACTERIZATION of 7-methyljuglone, 8,8′-dihydroxy-4,4′-dimethoxy-6,6′-dimethyl-2,2′-binaphthyl-1,1′-quinone, 2-methylnaphthazarin, mamegakinone and euclein[1] in the roots of *Euclea pseudebenus* E. Mey ex DC. has already been reported. In a continuing investigation of this plant, several other naphthoquinones have now been identified.

RESULTS AND DISCUSSION

From the light petroleum extract of the roots of *E. pseudebenus* two naphthoquinones were obtained. One, $C_{22}H_{14}O_6$, M^+ 374, m.p. 250–253°, has light absorption typical of a juglone [λ_{max} (EtOH) 218, 258, 436 nm; ν_{CO} (KBr) 1670, 1640, 1610, 1600 cm^{-1}] and is clearly a methyljuglone dimer. Its NMR spectrum shows three vinylic proton signals implying that the two methyljuglone units are linked from the aromatic ring of one of them to the quinonoid ring of the other, which is also supported by its MS. The most important ions resulting from the fragmentation of the substance in the mass spectrometer are: *m/e* 376 (M + 2), 375 (M + 1), 374 (M), 357 (M-OH), 356 (M-H_2O), 345 (M-CHO), 331 (M-Me-CO), and 328 (M-H_2O-CO), together with those with *m/e* 163, 135, 134, 106 and 77. These last ions result from the cleavage of an internal quinonoid ring and can be accounted for by assuming that the compound has the structure referred to above. On this basis, the substance now isolated from *E. pseudebenus* could be either euclein[1] (**1**) or diospyrin[2,3] (**2**) but since its IR spectrum, after solution in $CHCl_3$ and evaporation of the solvent at room temperature, superimposes exactly on that of a sample of diospyrin, it follows that it is identical to this compound (**2**).

In a previous communication, we concluded that diospyrin and euclein were different[1] on the grounds of differences in the IR spectra and also because the MS of euclein showed some minor differences when compared with the MS of diospyrin recorded by Fallas and Thomson.[4] Nevertheless, the NMR and MS of euclein and diospyrin, run under the same

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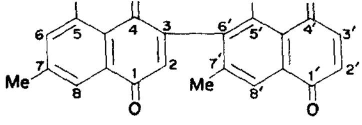
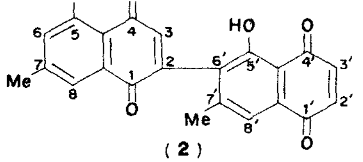
[1] FERREIRA, M A, ÁUREA CRUZ COSTA, M., CORREIA ALVES, A and LOPES, HELENA, M (1973) *Phytochemistry* **12,** 433

[2] KAPIL, R S and DHAR, M M (1961) *J. Sci Ind Res* (*India*) **20B,** 498

[3] SIDHU, G. S and PARDHASARADHI, M (1967) *Tetrahedron Letters* 1313, (1970) *Indian J Chem* **8,** 569



(1)



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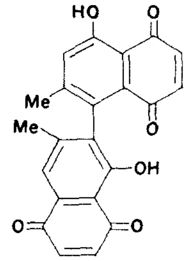
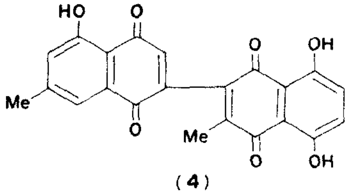


(3)



(4)

conditions and on the same apparatus, are completely identical. This discrepancy in the spectral analyses became comprehensible when it was observed by Mr. T J. Lillie in Professor Thomson's laboratory that other specimens of diospyrin all had the same IR spectrum as euclein, the exception being precisely the so-called authentic diospyrin* used by us for comparison with euclein.[1] However, when this diospyrin sample and that one obtained from *E pseudebenus* were dissolved in chloroform and the solvent allowed to evaporate, the materials so obtained then had the same IR spectrum as the other diospyrin samples and that of euclein Furthermore, the "euclein" dimethyl ether, prepared according to Sidhu and Pardhasaradhi,[5] has IR and NMR spectra which superimpose exactly on those of an authentic sample of diospyrin dimethyl ether

We were, therefore, misled by the solid state IR spectrum of the "authentic diospyrin" we used for comparison, which shows differences arising from the different crystal forms and we now recognize that euclein and diospyrin are the same compound The second substance isolated from the light petroleum extract of *E. pseudebenus* roots, $C_{22}H_{14}O_6$, $M^+ = 374$, m p. 230°, λ_{max} (EtOH) 219, 255, 434 nm, λ_{max} (EtOH/OH$^-$) 232, 298, 574 nm, ν_{CO} 1665, 1644, 1600, 1590 cm^{-1}, proved to be identical with an authentic sample of isodiospyrin[4] (**3**) (TLC, m m p, UV, IR, MS).

The powdered roots were afterwards extracted with chloroform, from which mamegakinone and diospyrin, along with four other substances, A_1, A_2, B_1 and B_2, were isolated by column chromatography and preparative TLC. A_1 was not identified but A_2, $C_{22}H_{14}O_7$, m.p 255–260°, λ_{max} ($CHCl_3$) 256, 284 (*sh*), 490, 524, 562 nm, ν_{CO} 1670, 1645, 1625, 1610, 1575 cm^{-1}, proved to be identical to 8′-hydroxydiospyrin[6] (**4**) (TLC, IR, MS).

B_1, $C_{22}H_{14}O_7$, m.p. > 340°, $[\alpha]_D = 0°$, λ_{max} ($CHCl_3$) 254, 298, 436, 484, 514, 553, ν_{CO} 1665, 1605 cm^{-1}, proved to be identical (IR, UV, NMR, MS, TLC) with racemic 8′-hydroxyisodiospyrin, a substance already isolated by us from the root bark of *Diospyros*

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* We thank Professor R H Thomson for kindly sending us this information

[4] FALLAS, A L and THOMSON, R H (1968) *J Chem Soc* (C), 2279

[5] SIDHU, G S and PARDHASARADHI M (1967) *Tetrahedron Letters* **14**, 1313

[6] MUSGRAVE, O C and SKOYLES, D forthcoming publication

lycioides, subsp. *sericea*.[7] B_2, appears to be a bisnaphthoquinone formed from 7-methyljuglone and methylnaphthazarin, but it could not be further identified

EXPERIMENTAL

Dried powdered roots of *Euclea pseudebenus** (800 g) were extracted with petrol (b p 50–70°) and $CHCl_3$ (Soxhlet) and then repeatedly macerated in $CHCl_3$ at pH 2–3 (with added HCl) The residue from the petrol extract (1 5%) was chromatographed on a column of silicic acid Elution with C_6H_6–$CHCl_3$ (1 9) removed three substances (TLC), and these were rechromatographed. Elution with C_6H_6 afforded mamegakinone (TLC, IR, m.p) and diospyrin (250 mg), m p 250–253°, λ_{max} (EtOH) 218, 256, 436 nm, λ_{max} (EtOH/OH^-) 230, 255 (*sh*), 290, 568 nm, ν_{max} (KBr) 1670, 1640, 1610, 1600, 1563 (*sh*), 1490, 1450, 1380, 1360 (*sh*), 1290, 1255, 1200, 1170, 1130, 1090, 1050, 1030 (*sh*), 990 (*sh*), 980 (*sh*), 925, 910 (*sh*), 865, 850, 830, 815 (*sh*), 780, 770 (*sh*), 760 (*sh*), 730, 705, 675 cm^{-1} After dissolution in $CHCl_3$ and the solvent allowed to evaporate, ν_{max} (KBr) 1660, 1648, 1615, 1600, 1565, 1485, 1445, 1385, 1360, 1335, 1260, 1210, 1160, 1130, 1090, 1050, 1000 (*sh*), 980 (*sh*), 915, 880, 855, 810, 785 (*sh*), 755, 710 cm^{-1} $C_{22}H_{14}O_6$ requires, M = 374 0790, found, M = 374 0794 Further elution of the same column with C_6H_6–$CHCl_3$ (9 1 and 9 2) extracted two substances These solutions were combined, concentrated and submitted to preparative TLC on silica gel in $CHCl_3$–C_6H_6 (4 1) Two bands were obtained The upper band was rechromatographed on a column of silicic acid in $CHCl_3$ and gave isodiospyrin (20 mg) m p. 230°, λ_{max} (EtOH) 219, 255, 434 nm, λ_{max} (EtOH/OH^-) 232, 298, 574 nm, ν_{max} (KBr) 1665, 1640, 1600 (*sh*), 1590 (*sh*), 1440, 1420, 1375 (*sh*), 1360, 1340, 1280, 1240, 1205, 1155, 1100, 1050, 1030, 990 (*sh*), 850, 820, 750, 700 (*sh*), 650 cm^{-1} $C_{22}H_{14}O_6$ requires, M = 374 0790, found M = 374 0786 The $CHCl_3$ solution from the Soxhlet extraction afforded 7-methyljuglone and 2-methylnaphthazarin (TLC, IR, UV-V, m p)

The extracts resulting from maceration in $CHCl_3$ were combined and evaporated to dryness The residue (0 23%) was chromatographed on a column of silicic acid Elution with C_6H_6-$CHCl_3$ (4 1, 7 3, 3 2, 1 1) gave red coloured solutions which were combined and submitted to preparative TLC on silica gel in $CHCl_3$ Three bands were obtained The upper band afforded mamegakinone, the lower band diospyrin, and the middle one, after repeated preparative TLC on silica gel in $CHCl_3$, separated into four bands, designated A_1, A_2, B_1 and B_2 according to their decreasing R_f values Each band was eluted with $CHCl_3$, and the solutions were passed through a silicic acid column and conc under reduced pressure

Substance A_1, m p 220/225°, λ_{max} ($CHCl_3$) 242, 256, 290 (*sh*), 460 (*sh*), 494, 523, 560 nm, ν_{max} (KBr) 1670 (*sh*), 1645, 1610, 1575 (*sh*), 1460, 1405, 1375, 1270, 1170 (*sh*), 1090, 1020 (*sh*), 950 (*sh*), 900, 870 (*sh*), 800 cm^{-1} Substance A_2, *8′-hydroxydiospyrin, m p 255–260°, λ_{max} ($CHCl_3$) 256, 284 (sh), 460 (sh), 492, 524 562 nm, ν_{max}* (KBr) 1670, 1645, 1625, 1616, 1575, 1455, 1410, 1375, 1320, 1270, 1230, 1200, 1170, 1100, 1075, 1040, 990, 870, 840, 800, 780 cm^{-1} $C_{22}H_{14}O_7$ requires M = 390 07385, found, M = 390 07396 Substance B_1, racemic 8′-hydroxyisodiospyrin m p > 340°, λ_{max} ($CHCl_3$) 254, (log ϵ = 4 11), 298 (log ϵ = 3 87), 436 (log ϵ = 3·80), 484 (log ϵ = 3 82), 514 (log ϵ = 3 79), 553 (log ϵ = 3 55) nm, ν_{max} (KBr) 1665 (*sh*), 1645, 1605, 1563 (*sh*), 1455, 1400, 1360, 1335, 1285, 1235 (*sh*), 1220, 1195, 1145, 1115, 1040, 950, 910, 895, 860 (*sh*), 805, 790 (*sh*), 770 (*sh*), 745 (*sh*), 730, 690 *cm*$^{-1}$ $C_{22}H_{14}O_7$ requires M = 390 0738, found M = 390 0735 Its CD curve shows that it is optically inactive Substance B_2, m p 260–270°, λ_{max} ($CHCl_3$) 244, 262 (*sh*), 310 (*sh*), 354, 454 (*sh*), 486, 514, 552 *nm*, ν_{max} 1680, 1640, 1610, 1580, 1455, 1415 (*sh*), 1410 (*sh*), 1360, 1335, 1320, 1290, 1270 (*sh*), 1245, 1210, 1180, 1140, 1080, 1050 (*sh*), 1015, 995, 950, 910 (*sh*), 870 (*sh*), 830, 790, 760 (*sh*), 705 *cm*$^{-1}$ $C_{22}H_{14}O_7$ requires, M = 390 07385, found, M = 390 07396 *Diospyrin dimethyl ether* "euclein" (25 mg) in $CHCl_3$ (5 ml) was shaken for 6 days at room temperature with AgO (250 mg) and MeI (1 ml) The dimethyl ether (12 mg) crystallized in yellow needles, m p 260–263° (from petrol–CH_2Cl_2) which proved to be identical to an authentic sample of diospyrin dimethyl ether ν_{max} (KBr) 1655, 1595, 1580, 1450, 1345, 1330, 1302, 1290, 1275, 1260, 1160, 1130, 1090, 1070, 1045, 1010 (*sh*), 950, 930, 920, 852, 820, 805, 780, 745, 710 (*sh*), 675 (*sh*), 660 (*sh*) Its 100 MHz NMR spectrum ($CDCl_3$) comprises singlets at δ 7 81 (1H, Ar-H), 7 59 (1H, Ar-H), 7 15 (1H, Ar-H), 6 87 (2H, Q-H), 6 76 (1H, Q-H), 4 02 (3H, OMe), 3·68 (3H, OMe), 2 48 (3H, Me) and 2 28 (3H, Me) After shaking with D_2O the NMR spectrum showed no alteration $C_{24}H_{18}O_6$ requires, M = 402 1098, found, M = 402 1103

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* Collected at Giraul, Angola; herbarium specimens are kept in the Institute for Scientific Research, Luanda

[7] Correia Alves, A Áurea Cruz Costa, M and Ferreira, M A (1973) *An Fac Fárm Porto* **33,** 5