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DESOXYCORDIFOLINIC ACID FROM *NAUCLEA DIDERRICHII*

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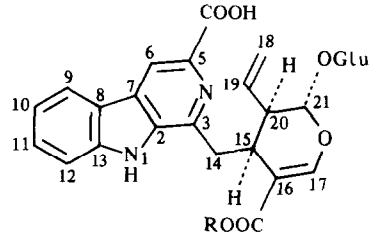
Key Word Index—*Nauclea diderrichii*; Rubiaceae; heartwood; alkaloids; β-carboline; desoxycordifolinic acid.

Abstract—The heartwood of the stems of *Nauclea diderrichii* yielded as its major alkaloid the parent diacid of desoxycordifoline, which has been named desoxycordifolinic acid.

INTRODUCTION

Previous work on the stems of *Nauclea diderrichii*, a large evergreen timber tree common in the rain forests of west tropical Africa, has shown the plant to be capable of elaboration of simple β-carbolines [1–6], in common with other species of the Rubiaceae, and a seco-iridoid [7]. A series of pyridines and β-carboline-pyridines has also been reported from the species [1–6], after extraction procedures which utilized ammonia; the presence of these in the original plant material has been questioned [8]. We have re-examined the heartwood, avoiding the use of ammonia and strong acids. The plant material was authenticated as described previously [7].



1a R = Me
1b R = H

RESULTS AND DISCUSSION

The powdered heartwood was subjected only to extraction with methanol and the methanol extract treated according to Brown and Warambwa [9] to achieve separation into neutral, basic and amphoteric fractions. The major alkaloid, and the only one so far obtained from the wood in sufficient quantity for identification, came from the amphoteric fraction after gel permeation chromatography, and was obtained crystalline from aqueous acetonitrile. UV data were consistent with a fully aromatic β-carboline structure and IR showed conjugated carbonyl as well as hydroxyl absorption. The 300 MHz ^{1}H NMR spectrum was of very high quality and showed only one misleading resonance: we at first interpreted a signal at *ca* δ11.9 as being caused by a carboxylic acid proton, but later work with model compounds showed that it was almost certainly attributable to the indolic N–H. The remainder of the spectrum allowed us to go some way to a structural assignment which will be explained by reference to the numbering in structure **1**. The β-carboline C-ring was represented by a singlet at δ 8.77, showing that the 5-position was substituted. There was a doublet a δ 8.36, a two-proton multiplet at *ca* 7.6 and a triplet at 7.30 showing that the benzenoid ring of the β-carboline was unsubstituted. There was clearly a substituent at C-3, and several resonances suggested that this was a seco-iridoid moiety similar to secologanin. There was a singlet at δ 7.50 corresponding to H-17 and a doublet at 5.56 for H-21. The vinyl group of secologanin-like iridoids was indicated by a doublet at δ 4.9 (J = 10 Hz), a doublet at 4.79 (J = 17 Hz), and the olefinic proton to which these were, respectively, *cis* and *trans* coupled appeared as a double triplet at 5.70.

A doublet at δ 4.59 was interpreted as the anomeric proton of a sugar, and this was supported by complex absorption in the range 2.9–3.8. There was a broad multiproton exchangeable peak at *ca* δ 5. The ^{13}C NMR spectrum showed typical resonances for a β-glucoside [10], as well as resonances for 21 other carbons, including two carbonyls, which could all be rationalized in terms of a structure similar to desoxycordifoline, **1a** [9]. On this basis all the ^{13}C NMR data could be assigned, with the proviso that some of the β-carboline assignments are uncertain. Our own data on model compounds do not support all the assignments for these carbons made by either of two earlier groups [11, 12], which are themselves in some respects contradictory. The assignments given in the Experimental are only quoted where there is definite agreement.

Methylation of the alkaloid with diazomethane gave a compound with two *O*-methyl resonances in the ^{1}H NMR spectrum, confirming the presence of two carboxylic acid groups, and implying that the original carboxyl resonances were included in the broad exchangeable peak at *ca* δ 5. The dimethyl ester of the new alkaloid proved to be identical in all respects with an authentic sample of desoxycordifoline methyl ester [9], allowing structural assignment of the new alkaloid, which we have called desoxycordifolinic acid **1b**.

After acetylation and methylation, desoxycordifolinic acid was compared with desoxycordifoline methyl ester tetra-acetate, again confirming identity. It was also poss-

ible, partially with the aid of decoupling, to assign all the peaks in the 300 MHz ^{1}H NMR spectrum of the double derivative, including the sugar protons. All the mass spectral, IR and UV data are in accord with the proposed structure (see Experimental).

It may be noted that the isolation of a 5-carboxy alkaloid of this type from a species of *Nauclea* does not support the taxonomic separation of the subtribe Adininae from the subtribe Naucleinae [13]. It is clear from the present work that such alkaloids are not restricted to Adininae. It is also possible that the adoption of suitable extraction procedures will show that the retention of glucose, a major characteristic of Adininae alkaloids [13], may be a feature of Naucleinae alkaloids as well. In this respect, the use of ammonia and/or strong acid with highly labile seco-iridoid moieties not only ensures the production of artefacts but may also obscure the true nature of the alkaloids present naturally, by removing glucose residues.

So far we have not identified any pyridine-containing alkaloids from *N. diderrichii* but we are continuing our efforts to identify the tetrahydro-β-carbolines which have been isolated in small quantities from the same extract, and which again appear to retain sugar moieties.

EXPERIMENTAL

Powdered heartwood of *N. diderrichii* (De Wild.) Merr. (*Sarcocephalus diderrichii* De Wild) (4 kg) was exhaustively percolated with cold MeOH. The extract was evaporated to give a brown powder (217 g). The powder (100 g) dissolved in MeOH (300 ml) and passed through a column of Amberlyst A15 resin, eluting first with MeOH and then with 10% Et_3N in MeOH, the latter giving a brown powder (30 g) after evaporation. This material was passed through a column of Amberlyst A26 resin, eluting first with MeOH to give a basic fraction (8.2 g) and then with 10% HOAc in MeOH to give an 'amphoteric' fraction (21 g). The 'amphoteric' fraction (10 g) was passed through a Sephadex LH20 column, eluting with MeOH. Contiguous fractions with UV and TLC indications of an alkaloidal nature were bulked to give a dark brown powder (5.9 g). This powder (0.4 g) was shaken with 80% aq. MeCN (25 ml), the clear soln decanted and left in the dark for 48 hr. Desoxycordifolinic acid was obtained as brown rosettes (56 mg), mp 206–208° (dec.). $[\alpha]_D$ −45.7° (MeOH; *c* 0.126) $\Delta\varepsilon_{242}$ +2.09, $\Delta\varepsilon_{267}$ −11.9, $\Delta\varepsilon_{290}$ −2.28, $\Delta\varepsilon_{303}$ −1.22, $\Delta\varepsilon_{376}$ −1.25 (MeOH; *c* 0.160) IR ν_{max}^{KCl} cm^{-1}: 1650 (>C=O). UV λ_{max}^{EtOH} nm: 216, 236, 272, 306sh, 354. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.93 (1H, *s*, NH), 8.77 (1H, *s*, H-6), 8.36 (1H, *d*, *J* = 8 Hz, H-9) 7.62 (1H, *m*, H-12), 7.58 (1H, *m*, H-11), 7.50 (1H, *s*, H-17), 7.30 (1H, *t*, *J* = 7 Hz, H-10), 5. 7 (1H, *dt*, *J* = 10, 17 Hz, H-19), 5.56 (1H, *d*, *J* = 5 Hz, H-21), 4.90 (1H, *d*, *J* = 10 Hz, H-18a), 4.79 (1H, *d*, *J* = 17 Hz, H-18b), 4.59 (1H, *d*, *J* = 8 Hz, H-1), 3.69 (2H, *m*, H-6′ a,b), 3.45 (1H, *m*, H-15), 3.07–3.25 (6H, *m*, H-14a,b, H-2′, H-3′, H-4′, H-5′), 2.64 (1H, *m*, H-20). ^{13}C NMR (20.1 MHz, DMSO-d_6): δ 167.6 (*s*, COOH), 151.1 (*d*, C-17), 143.4 (*s*, C-3), 140.4 (*s*), 136.0 (*s*), 135.7 (*s*, C-5), 134.1 (*d*, C-19), 128.0 (*d*), 127.0 (*s*), 121.7 (*d*, C-9), 121.0 (*s*), 119.8 (*d*), 118.2 (*t*, C-18), 114.8 (*d*, C-6) 112.0 (*d*, C-12), 110.1 (*s*, C-16), 98.3 (*d*, C-1′), 95.4 (*d*, C-21), 77.1 (*d*, C-5′), 76.5 (*d*, C-3′), 72.9 (*d*, C-2′), 69.9 (*d*, C-4′), 60.9 (*t*, C-6′), 43.3 (*d*, C-20), 32.8 (*t*, C-14), 30.4 (*d*, C-15). EIMS, 70 eV *m/z* (rel. int.): 332 [M − glu − COOH]$^+$ (3), 314 (1), 288 (6), 270 (6), 259 (3), 243 (4), 226 (5), 205 (3), 182 (23), 179 (1), 154 (4), 126 (15), 97 (15), 44 (100). CIMS (NH_3) *m/z* (rel. int.): 333 (36), 315 (12), 289 (72), 271 (13), 227 (100), 198 (11), 183 (33), 180 (81), 162 (13), 144 (39), 127 (79). Found C, 56.0; H, 5.2; N, 4.8%. $C_{27}H_{28}N_2O_{11}\cdot H_2O$ requires C, 56.4; H, 5.2; N, 4.9%. Treatment of a MeOH soln of desoxycordifolinic acid with CH_2N_2–Et_2O followed by prep. TLC on Si gel gave di Me desoxycordifolinate mp 144–146°, mmp with Me desoxycordifoline, 145–147°, UV, IR, ^{1}H NMR and MS in accord with structure. Acetylation (Ac_2O, pyridine) followed by methylation and prep. TLC on Si gel gave diMe desoxycordifolinate tetra-acetate, mp 102–104°, mmp with methyl desoxycordifoline tetra-acetate, 101–104°. UV λ_{max}^{MeOH} nm: 235, 274, 318, 337, 350. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750, 1680. ^{1}H NMR (300 MHz, $CDCl_3$): δ10.82 (1H, *s*, NH), 8.83 (1H, *s*, H-6), 8.22 (1H, *d*, *J* = 8 Hz, H-9), 7.72 (1H, *d*, *J* = 8 Hz, H-12), 7.63 (1H, *t*, *J* = 8 Hz, H-11), 7.54 (1H, *s*, H-17), 7.38 (1H, *t*, *J* = 8 Hz, H-10), 5.89 (1H, *dt*, *J* = 10, 17 Hz, H-19), 5.50 (1H, *d*, *J* = 5 Hz, H-21), 5.23 (1H, *d*, *J* = 17 Hz, H-18a), 5.23 (1H, *t*, *J* = 8.5 Hz, H-3′), 5.22 (1H, *d*, *J* = 10 Hz, H-18b), 5.06 (1H, *t*, *J* = 10 Hz, H-4′), 4.90–4.91 (2H, *m*, H-1′, H-2′), 4.30 (1H, *dd*, *J* = 12, 4.5 Hz, H-6′a), 4.15 (1H, *dd*, *J* = 12, 2 Hz, H-6′b), 4.05 (3H, *s*, OMe), 3.90 (3H, *s*, OMe), 3.76 (1H, *m*, H-5′), 3.69 (1H, *d*, *J* = 14 Hz, H-14a), 3.35 (1H, *dd*, *J* = 14, 10 Hz, H-14b), 3.23 (1H, *dd*, *J* = 10, 5 Hz, H-15), 2.67 (1H, *dt*, *J* = 10, 5 Hz, H-20), 1.97, 1.98, 2.02, 2.10 (12H, 4*s*, 4 × COMe). EIMS, 70 eV *m/z* (rel. int.): 752.2449 [M]$^+$. Calc. for $C_{37}H_{40}N_2O_{15}$: 752.2428 (5), 421.1398; calc. for $C_{23}H_{21}O_6$: 421.1399 (19), 405.1449; calc. for $C_{23}H_{21}N_2O_5$: 405.1450 (27), 376 (5), 335 (12), 331 (6), 240 (27), 169 (57), 165 (6), 139 (5), 127 (14), 115 (12), 109 (44), 85 (58), 83 (100), 47 (18), 43 (86).

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