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NEOFLAVANOIDS OF *DALBERGIA MELANOXYLON**

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Key Word Index—*Dalbergia melanoxylon*; Leguminosae; neoflavanoids; benzophenones; 4-phenylcoumarins; 4-methoxydalbergiones; 2-cinnamylphenol; 3-cinnamylflavan.

Abstract—The heartwood of *Dalbergia melanoxylon* has yielded new examples of known neoflavanoid types. The isolates included 4-phenylcoumarins, (*S*)-4-methoxydalbergiones and benzophenones which have the corresponding oxygenation pattern. The structures of the extractives have been examined by physical methods, and confirmed by conversion to known compounds or by independent synthesis. A 2-cinnamylphenol (**6**) and 3-cinnamylflavan (**7**) were the only products in a condensation reaction in the attempted synthesis of the 4-methoxydalbergione (**1b**).

INTRODUCTION

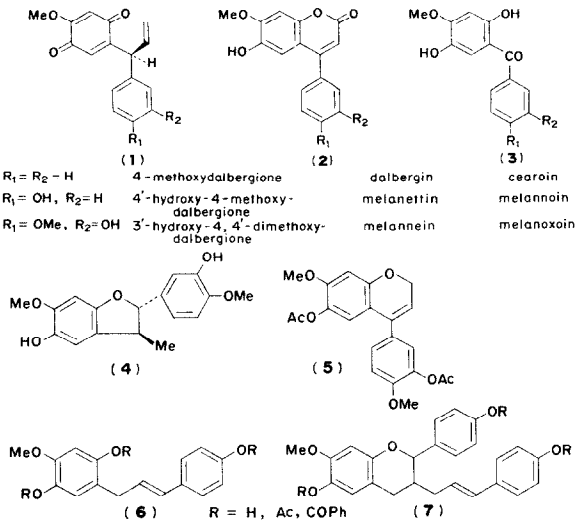
In a continuation of our studies of the extractives of *Dalbergia melanoxylon* Guill et Perr. (African Blackwood, Senegal Ebony, Mozambique Ebony) (Leguminosae–Lotoideae), new examples of known neoflavanoids have been isolated. Previous analysis [1] led to isolation of (*S*)-4-methoxydalbergione (**1a**), (*S*)-4′-hydroxy-4-methoxydalbergione (**1b**), melannein (**2c**) and melanoxin (**4**). The presence of many neoflavanoids and lack of isoflavanoids supports the placement of this species in the species-series *Dalbergiae pantropicales* [2]. To date, *D. melanoxylon* has yielded six neoflavanoids which may be subdivided into three distinct groups based on the oxygenation pattern of ring-B. The corresponding benzophenones were also present. The major component in the extract was melanoxin (**4**)—a 2,3-dihydrobenzofuran.

RESULTS AND DISCUSSION

The acetone extract of heartwood was fractionated and afforded three distinct groups of neoflavanoids. Those of greatest abundance were the most highly oxygenated series. The 4-methoxydalbergiones present included the known (*S*)-4-methoxydalbergiones [3] (**1a**) and (*S*)-4′-hydroxy-4-methoxydalbergione [3] (**1b**) and a new optically active dalbergione $C_{17}H_{16}O_5$. Its NMR spectrum revealed the now characteristic ABCX pattern for the $>CH–CH=CH_2$ group which permitted the assignment of chemical shifts to the four protons. The 3-H and 6-H signals were evident as were the signals for methoxyl groups (6·08; 6·15τ) and an hydroxyl group (4·4τ). The oxygenation pattern in the B-ring was confirmed by conversion of the dalbergione to the known 4-phenylcoumarin, melannein. This conversion [4] was achieved by action of *N*,*N*-dimethylaminopyridine in $CHCl_3$ and subsequent acetylation to give melannene diacetate (**5**). The neoflavene (**5**) with CrO_3–C_5H_5N gave a quantitative yield of melannein diacetate. ORD analysis identified the chiral centre as having a (*S*)-configuration and the structure of the dalbergione is depicted in the formula (**1c**).

The unknown coumarin $C_{15}H_{14}O_6$ (**2b**), for which the name melanettin is suggested, showed similarities in its spectroscopic data with those of dalbergin (**2a**) [5] and melannein (**2c**) [1b]. The formation of a diacetate (ν_{CO} 1744; τ 7·67, 7·61) confirmed the presence of two phenolic hydroxyl groups. The aromatic protons in the NMR spectrum of the diacetate had signals for an A_2B_2

* Part 12 in the series "*Dalbergia* species".



system and two singlets which are characteristic of the 5- and 8-protons in the 6,7-disubstituted A-ring. The mass spectrum of the diacetate showed loss of two CH_2CO fragments and subsequent loss of 28 mass units to give *m/e* 256 signal. The further loss of a methyl radical was registered but the lower mass ions were not abundant and no metastable peaks were apparent.

As insufficient material was available for degradation a synthesis of the coumarin (**2b**) was undertaken. Two approaches to the synthesis were planned (i) the cyclization of (±)-4-methoxydalbergione (**1b**) and subsequent oxidation of the resultant neoflavene and (ii) a Perkin condensation with benzophenone (**3b**). The only reaction products from the attempted synthesis of the 4-methoxydalbergione (**1b**) via cinnamylation of the respective phenol in the presence of ascorbic–citric acids were 2',4,5'-trihydroxy-4'-methoxybenzylstyrene (**6**, R = H) and 4',6-dihydroxy-3-(4-hydroxycinnamyl)-7-methoxyflavan (**7**, R = H) 3-Cinnamylflavans have been isolated in a number of phenol-cinnamyl alcohol condensations [6]. The compound (**6**) and (**7**) showed characteristic spectroscopic data. The feature of additional signals for the X_2 protons in the ABX_2 system of the benzylstyrene [7] was present. The 2,5,4'-trihydroxy-4-methoxybenzophenone (**3b**), which co-occurred with coumarin (**2b**), was prepared also by acylation of 1,2,4-trimethoxybenzene by 4-methoxybenzoyl chloride and subsequent partial demethylation of the product with HI, was fused with NaOAc and Ac_2O. The synthetic and natural coumarins were identical.

The three benzophenones cearoin (**3a**) [8] and two new members of the class for which the names melannoin (**3b**) and melanoxoin (**3c**) are suggested were found to be present. The structure of each was confirmed by comparison with authentic samples. Cearoin [8] has been isolated previously from two other species *D. cearensis* and *D. miscolobium* but the benzophenones (**3b**) and (**3c**) are new natural products. The presence of melannoin (**3b**) was detected by GLC.

All biosynthetic schemes proposed for the formation of the neoflavanoids of *Dalbergia* and its close relative *Macherium* are based on comparative phytochemistry [2]. Limitation is placed on an assessment of the sequences of reaction steps by which the individual compounds arise from precursors. The isolation of three 4-methoxydalbergiones, three 4-phenylcoumarins and the corresponding three benzophenones points to the completion of the oxygenation pattern prior to their interconversion via bio-oxidative or bio-reductive sequences. The major isolate is the (2*S*, 3*S*)-2,3-dihydrobenzofuran, melanoxin. In view of the co-occurrence of the series of neoflavanoids the presence of obtusafuran and the 4'-hydroxydihydrobenzofuran and the neoflavenes is suspected. To date, the search for these compounds is without success.

EXPERIMENTAL

Unless otherwise stated, the following generalizations apply. Mp's were determined on a Kofler hot-stage apparatus. IR spectra were measured for KBr discs; UV spectra were determined in MeOH and 60 MHz NMR spectra in $CDCl_3$ (tetramethylsilane as internal reference). Only significant bands in IR and NMR spectra are quoted. Mass spectra were obtained with an A.E.I. MS 902 (direct inlet) instrument. Optical rotations were measured on a Perkin–Elmer Model 141 Polarimeter. Separations by column chromatography were carried out using Merck Si gel. Merck Kiesegel HF_{254} and $PF_{254+366}$ were used for thin- and thick-layer chromatography (TLC). During isolation processes the appropriate combination of fractions were determined by TLC. The TLC plates were examined with UV illumination and by spraying with chlorosulphonic acid in HOAc.

Extraction of Dalbergia melanoxylon *Guill et Perr. heartwood.* A portion (20 g) of the EtOH–Me_2CO extract (**1a**) was chromatographed (Si gel; 800 g) successively with $CHCl_3$ and $CHCl_3$–Me_2CO (9.5:0.5; 9:1; 1:1) as eluent. This procedure was repeated three times. The appropriate combination of fractions (i–vi) was determined by examination of their spectra and TLC behaviour. The products were purified by PLC. The relevant bands on TLC plates (numbered in order of decreasing R_f values) were eluted with Me_2CO. Fraction (i) [eluent: $CHCl_3$(0·5l)] was a pungent smelling oil. The small quantity

of material precluded further analysis. Fraction (ii) [eluent $CHCl_3$ (1l)] was purified by TLC (eluent C_6H_6) and gave (*S*)-4-methoxydalbergione (**1a**), mp 115° (lit. [3] 118°), yellow needles from di-isopropyl ether; and 2,5-dihydroxy-4-methoxybenzophenone (cearoin) (**3a**), mp 182–184° (lit. [7] 188°), yellow amorphous solid from C_6H_6–light petrol (bp 60–80°). (Found: C, 68·7; H, 5·18. Calc. for $C_{14}H_{12}O_4$ C, 68·84; H, 4·95%). This compound gave an olive green colour with ethanolic $FeCl_3$. Fraction (iii) [eluent $CHCl_3$ (1l)] afforded melannein (**2c**) (300 mg) mp 221–223° (lit. [1b] 221–223°) pale yellow rhombs from EtOH. The mother liquor, a red oil, was purified by PLC (1% Me_2CO–$CHCl_3$; double development) giving (*S*)-3′-hydroxy-4,4′-dimethoxydalbergione (**1c**) (120 mg) mp 101–102°, rhombs from di-isopropyl ether (Found: C, 68·04; H, 5·12. $C_{17}H_{16}O_5$ requires C, 68·0; H, 5·35%); λ_{max} nm (ε) 230 (30900), 260 (38600); $\lambda_{max}^{MeOH-NaOMe}$ 243 (51700) 272 (38000) 301 (19500), 323 (13400); ν3465 cm^{-1} 1665 cm^{-1} 1640 cm^{-1}; $[\alpha]_D^{21°}$ −143·8 ($CHCl_3$); NMR τ3·42 (*d*, *J* 1 Hz, 6-H) 4·0 (*s*, 3′-OH), 6·08, 6·15 (*s*, 2 × OMe); τ_A5·1 (*d*), τ_B4·7 (2 × t), τ_C4·95 (2 × t), τ_X3·8 (*m*) (*ABCX* system, J_{AX} 6·0 Hz, J_{BX} 9·5 Hz, J_{CX} 15 Hz; $J_{AB} = J_{AC} = J_{BC}$ = 1·3 Hz, $J_{A,6}$ 1·0 Hz). Fraction (iv) (eluent $CHCl_3$–Me_2CO 9·5/0·5) afforded melanoxin (**4**), mp 107–108° (lit. [1b] 107) needles from ligroin $[\alpha]_D^{21°}$ −49(Me_2CO). Fraction (v) (eluent: $CHCl_3$–Me_2CO/19:1), a brown oil which on addition of $CHCl_3$ afforded (*S*)-4′-hydroxy-4-methoxydalbergione (**1b**), mp 180–185° (lit. [3] 172–178°), orange rosettes from MeOH; $[\alpha]_D^{21°}$ −69·9 (Me_2CO). The filtrate following PLC (in $CHCl_3$–Me_2CO/9:1) gave four bands. The fast moving band (1) was further fractionated (PLC eluent: 2·5% Me_2CO–$CHCl_3$) and gave (*S*)-3′-hydroxy-4,4′-dimethoxydalbergione (**1c**), dalbergin (**2a**) and melanoxin (**4**); band (2) afforded (*S*)-4-hydroxy-4-methoxydalbergione; the residue from band (3) was examined and on PLC (eluent: $CHCl_3$–Me_2CO/19:1) gave 2,5,3′-*trihydroxy*-4,4′-*dimethoxybenzophenone* (**3c**), mp 232–234°, yellow amorphous solid from C_6H_6–light petrol (bp 60–80°). (Found: C, 62·28; H, 5·11. $C_{15}H_{14}O_6$ requires C, 62·06; H, 4·86%); λ_{max} nm (ε) 248 (18600) 290 (12800) 363 (12600); $\lambda_{max}^{MeOH-NaOMe}$ 254 (33200) 287 (14000) 365 (7700); ν_{max}2330 cm^{-1} 1630 cm^{-1}. The slow moving material band (4) afforded melannein (**2c**) and 2,5,4′-trihydroxy-4-methoxybenzophenone (**3b**). The presence of the latter compound was confirmed by GLC analysis of acetylated band (4) (on a column 2·8% SE 30) and comparison with an authentic sample of 2,5,4′-triacetoxy-4-methoxybenzophenone. The retention time of the benzophenone triacetate at 225° (N_2 = 45 lbs, H_2 = 12 lbs p.s.i., O_2 30 lbs/sq in.) was 5 min 46 sec. Fraction (vi) (eluent: $CHCl_3$–ME_2CO/9:1) afforded a brown solid (125 mg). Purification by acetylation gave 6,4′-*diacetoxy*-7-*methoxy*-4-*phenylcoumarin*, mp 147–148° from EtOH (Found: M (mass spectrum) 368, *m/e* 326 (85%), 284 (100%), 266 (28%). $C_{20}H_{16}O_7$ requires M^+ 368). Hydrolysis of this acetate gave 6,4′-dihydroxy-7-methoxy-4-phenylcoumarin (**2b**), mp 233–234°, amorphous solid from Me_2CO. Found M (mass spectrum) 284. $C_{16}H_{12}O_5$ requires M, 284); λ_{max} nm (ε) 230 (31900) 263 (12500) 312 (18900) 350 (16400); ν_{max} 3430 cm^{-1} 1660 cm^{-1}.

Characterization and reactions of the natural products isolated from Dalbergia melanoxylon. (S)-3′-*Acetoxy*-4,4′-*dimethoxydalbergione quinol diacetate*. Dalbergione (**1b**) (100 mg) was dissolved in CH_2Cl_2 and shaken with aq sodium dithionite (30%). The CH_2Cl_2 soln was poured into Ac_2O (6 ml) and C_5H_5N (2 ml) and the mixture was allowed to stand for 12 hr. The *triacetate* was crystallized from di-isopropyl ether in needles (83 mg), mp 99–100°; $[\alpha]_D^{21°}$ −14·3° ($CHCl_3$) (Found: C, 64·66; H, 5·6. $C_{23}H_{24}O_8$ requires C, 64·48; H, 5·6%). λ_{max} nm (ε) 225 (28300) 285 (7780); NMR τ 7·82 (*s*, 2-OAc 7·69) (*s*, 5-OAc, 3′-OAc), 6·14 (*s*, OMe), τ_A 5·25 (*d*) τ_B 4·8 (2 × t) τ_C 5·1 (2 × t), τ_X 3·9 (*m*) (*ABCX* system J_{AX} 5·5; J_{BX} 10; J_{CX} = 16 Hz, Ar_2C (H$_A$) ◀ C(–H$_X$) = C(H$_B$H$_C$) 3·0–3·3 (*m*, ring B protons).

2,5,3′-*Triacetoxy*-4,4′-*dimethoxybenzophenone*. Acetylation of the benzophenone (**3c**) (by Ac_2O and C_5H_5N method) gave a *triacetate* which crystallized from EtOH as needles, mp 155–157°. Found: C, 60·1; H, 5·03, M (mass spectrum) 416 $C_{21}H_{20}O_9$ requires C, 60·6; H, 4·84 M^+ 416) λ_{max} nm (ε) 288 (49500), 226 (57800); NMR τ 7·98 (*s*, 2′-OAc) 7·66 (*s*, 5 OAc, 3′-OAc), 6·06 (*s*, OMe), 3·1 (*s*, 3-H), 2·95, 2·25 (*q*, *J* 2·0, 7·0 Hz 5-H, 6′-H) 2·6 (*s*, 6-H), 2·4 (*d*, *J* 2·0 Hz 2′-H) ν_{max} 1760 cm^{-1}; 1655 cm^{-1}.

5,3′-*Diethoxy*-2-*hydroxy*-4,4′-*dimethoxybenzophenone*. The benzophenone (40 mg) was refluxed with Et_2SO_4 (45 mg), K_2CO_3 (1 g) in $(Me)_2CO$ (8 ml) for 6 hr. The inorganic salts were removed, the filtrate washed and evaporated. The residue was purified by TLC (developer Et_2O–light petrol (bp 40–60°) 4:1). The diethyl derivative (22 mg) was crystallized from MeOH as yellow needles mp and mmp (with an authentic sample (**1b**)) 121°.

Synthesis of 2,5,4′-*trihydroxy*-4-*methoxybenzophenone*. Friedel–Crafts acylation of 1,3,4-trimethoxybenzene by 4-methoxybenzoyl chloride afforded 2-hydroxy-4,5,4′-trimethoxybenzophenone as needles from C_6H_6–$CHCl_3$ mp 124–125° (lit. mp 127–128°). Addition of HI (18 ml) and Ac_2O (12 ml) to the above 2-hydroxy-4,5,4′-trimethoxybenzophenone (2 g) gave a mixture 1·05 g) which was fractionated (Si gel column). The eluent was $CHCl_3$. The 2,5,4′-*trihydroxy*-4-*methoxybenzophenone* and 2,5,4,4′-*tetrahydroxybenzophenone* were isolated. The latter compound was unstable and was acetylated directly. The *tetraacetate* crystallized from EtOH in clusters of needles mp 132–134° (Found: C, 60·96; H, 4·34. $C_{21}H_{18}O_9$ requires C, 60·87; H, 4·38%).

2,5,4′-*Trihydroxy*-4-*methoxybenzophenone* (**3b**), mp 228–229·5°, yellow plates form C_6H_6–Me_2CO. Found: C, 64·8; H, 4·7. $C_{14}H_{12}O_5$ requires C, 64·6; H, 4·6%), NMR [$(CD_3)_2CO$] τ 6·45 (*s*, OMe) 3·78 (*d*, *J* 1·0 Hz, 3-H), 3·28 (*d*, *J* 1·0 Hz, 6-H), 3·2, 2·7 (*q*, *J* 7·0 Hz, A_2B_2 system -3·0 *s*, 2-OH), 2·9, 1·1 (broad *s* 5-OH, 4′-OH)

2,5,4′-*Triacetoxy*-4-*methoxybenzophenone*. Acetylation of the benzophenone (**3b**) (Ac_2O–C_5H_5N) gave needles from EtOH, mp 127–128° (Found: C, 61·9; H, 4·7. $C_{20}H_{18}O_8$ requires C, 62·17: H, 4·7%). NMR τ7·98 (*s*, 2-OAc), 7·66 (*s*, 2 × OAc), 6·06 (*s*, OMe), 3·1 (*s*, 3-H), 2·48 (*s*, 6-H), 2·08 (*q*, *J* 8·0 Hz A_2B_2 system).

Attempted synthesis of (±)-4′-*hydroxy*-4-*methoxydalbergione*. Cinnamylation of 4-hydroxy-4-methoxyphenol (500 mg) with 4-hydroxycinnamyl alcohol (500 mg) in ascorbic acid (125 mg) and citric acid (25 ml; 50%) was carried out at 60° for 20 hr. The soln was diluted and extracted with Et_2O which was subsequently washed, dried and evaporated. Oily residue was treated with Ac_2O (5 ml) and C_5H_5N (2 ml) and kept at 21° for 12 hr. The reaction mixture was poured on ice–HCl, and then extracted with Et_2O. Evaporation gave an oil (800 mg) which was fractionated by PLC (C_6H_6–EtOAc, 9:1; double development) Band (1) afforded 4′,6-*diacetoxy*-3-(4-*acetoxycinnamyl*)-7-*methoxyflavan* (**7** R = Ac) (250 mg) which was crystallised as needles from MeOH mp 144–145°. (Found: C, 70·2; H, 5·4. $C_{31}H_{30}O_8$ requires C, 70·2; H, 5·7%). ν_{max} 1755 cm^{-1} 1628, 1590 cm^{-1}. NMR τ_A 2·52 τ_B 2·92 (*q*, *J* 9·0 Hz, A_2B_2 system, cinnamylphenyl) τ_A 2·63 τ_B 2·77 (*q*, *J* 8·4 Hz A_2B_2 system, 2-Ph), τ 6·21 (*s*, OMe), 7·7 (*s*, 3 × OAc), 3·18 (*s*, 5-H), 3·4 (*s*, 8-H), 5·2 (*d*, *J* 4·5 Hz 2-H). λ_{max} nm (ε) 204 (5·3 × 10^4) 255 (2·72 × 10^4) 282 (sh) (8·65 × 10^3). Band (ii) gave 2′,4,5′-*triacetoxy*-4′-*methoxybenzylstyrene* (6; R = Ac) (460 mg) which was crystallized as needles from MeOH, mp

117–118°. (Found: C, 66·6; H, 5·5. $C_{22}H_{22}O_7$ requires C, 66·3; H, 5·6%). ν_{max} 1760 cm^{-1} 1619, 1507, 1500 cm^{-1}. NMR τ_A 2·58, τ_B 2·89 (*q*, *J* 8·4 Hz A_2B_2 system) τ_A 3·5, τ_B 3·6, τ_{X_2} 6·6 (J_{AB} 16·0 Hz, J_{AX} 5·4 Hz) τ 2·98 (*s*, 3-H) 3·21 (*s*, 6-H) 6·18 (*s*, OMe) 7·71 (*s*, 3 × OAc) λ_{max} nm (t) 206 (4·96 × 10^4) 2·56 (3·38 × 10^4). The *tribenzoate* (**6** R = COPh) crystallized as an amorphous solid from EtOH mp 104–105°. (Found: C, 77·4, H, 5·2. $C_{46}H_{36}O_8$ 77·08; H, 5·06%). ν_{max} 1738 cm $^{-1}$. λ_{max} nm (ϵ) 209 (ϵ 5·9 × 10^4) 2·31 (6·95 × 10^4) 252sh (3·78 × 10^4) 280 (1·41 × 10^4). The *tribenzoate* (**7**; R = COPh) crystallized as needles from EtOH mp 178–180°. (Found: C, 75·8; H, 5·0. $C_{37}H_{28}O_7$ requires C, 76·01; H, 4·84%). ν_{max} 1730 cm^{-1}.

Synthesis of 6,4′-diacetoxy-7-methoxy-4-phenylcoumarin. 2,5,4′-Trihydroxy-4-methoxybenzophenone (500 mg), fused NaOAc (500 mg) and Ac_2O (4 ml) were refluxed for 24 hr. The reaction mixture was thrown on ice–HCl and the ppt (500 mg) was purified by preparation TLC (developer C_6H_6–EtOAc, 9:1, developed 4×). 6,4′-Diacetoxy-7-methoxy-4-phenylcoumarin (60 mg) was crystallized from EtOH mp 149–150°. Found: C, 65·19; H, 4·51. $C_{20}H_{16}O_7$ requires C, 65·21; H, 4·38%). ν_{max} 1744 cm^{-1}, 1620 cm^{-1}, 1605 cm^{-1}. NMR τ 7·67, 7·61 (*s*, 2 × OAc), 6·01 (*s*, OMe), 3·66 (*s*, 3-H), 2·92 (*s*, 8-*H*), 2·75 (*s*, 5-H), 2·67, 2·41 (*q*, *J* 8·0 Hz A_2B_2 system).

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