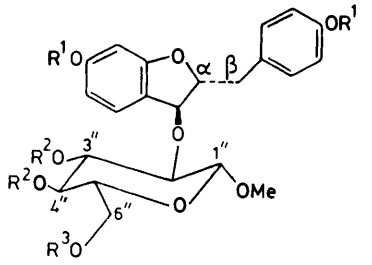
Neoraufuracin and Ambofuracin. Two Novel 2-Benzyl-2-*O*-(methyl-β-D-glucopyranos-2-yl)-*trans*-2,3-dihydrobenzofurans. Synthesis of the Methylated Aglycone

By **Jaco C. Breytenbach, Gerhardus J. H. Rall,** and **David G. Roux,*** Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300 Republic of South Africa
William E. Hull, Bruker Analytische Messtechnik GmbH, Silberstreifen, D-7512 Rheinstetten–Forchheim, West Germany

The structures of two novel 2-benzyl-3-*O*-(methyl-β-D-glucopyranos-2-yl)-*trans*-2,3-dihydrobenzofurans are elucidated using high resolution ^{1}H n.m.r., ^{13}C n.m.r., and field-desorption mass spectrometry, and also by synthesis of a derivative of the aglycone moiety common to both compounds.

SYSTEMATIC examination of the metabolites present in *Neorautanenia amboensis* Schinz for compounds which exhibit useful physiological activity has hitherto revealed a wealth of pterocarpans,[1] as well as a series of rotenoids [2] and isoflavanones. These isoflavonoids are the main constituents of the successive hexane and benzene extracts of this leguminous plant.

The final methanol extract of the bark contains (in addition to sugars, amino-acids, and five unidentified isoflavone glycosides) two novel 2-benzyl-3-*O*-(methyl-β-D-glucopyranos-2-yl)-*trans*-2,3-dihydrobenzofurans, neoraufuracin (1a) and ambofuracin (2a). Separation by counter-current distribution between ethyl acetate and water gives, among others, a fraction with a partition coefficient approaching unity (*cf.* Table, Experimental section) which, after column chromatography on silica gel, affords neoraufuracin (1a) and ambofuracin (2a). Both compounds are characterized by the purple colour

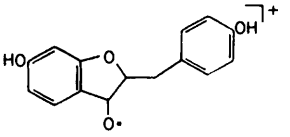


(1a) $R^1 = R^2 = R^3 = H$
(1b) R^1 = Me, $R^2 = R^3 = H$
(1c) R^1 = Me, $R^2 = R^3$ = Ac
(1d) $R^1 = R^2 = R^3$ = Ac
(2a) $R^1 = R^2 = H$, $R^3 = COCH_2CH_2CO_2Me$
(2b) $R^1 = R^2$ = Ac, $R^3 = COCH_2CH_2CO_2Me$

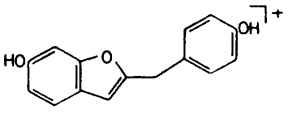
Relative stereochemistry only is indicated

they develop with the perchloric acid–iron(III) chloride spray reagent. Neoraufuraein (1a) is remarkably stable towards acid (HCl) and enzymic (α- and β-glucosidase) hydrolysis, but a positive Molisch test confirmed the presence of a carbohydrate moiety. Although the compound is crystalline, no suitable crystal for an *X*-ray analysis could be obtained, thus precluding assignment of the absolute configuration.

A molecular mass of **434** for neoraufuracin (1a) is obtained by field-desorption mass spectrometry, with substantial fragmentation to ions at *m/e* 257 and 240 which originate from the aglycone moiety. A molecular formula of $C_{22}H_{26}O_9$ for compound (1a) corresponding to the molecular mass, agrees with the carbon and hydrogen atom counts as deduced from the respective ^{13}C and ^{1}H n.m.r. spectra. Field-desorption mass spectrometry



m/e 257



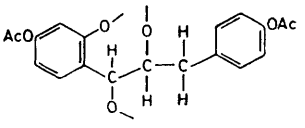
m/e 240

also shows the expected molecular mass of **644** for the penta-acetate, which is confirmed by elemental analysis consistent with the anticipated formula of $C_{32}H_{36}O_{14}$ for compound (1d).

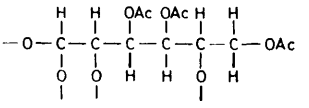
The 80 MHz ^{1}H n.m.r. spectrum of neoraufuracin (1a) exhibits aromatic ABX and AA_1BB_1 systems (*cf.* Experimental section), but the δ 2.0—4.0 region is exceedingly complex and reveals little about the heterocyclic nature of the compound. Much higher magnetic field strength is required for effective resolution of this region (see later). However, 80 MHz spectrometry assists in defining the nature of the functional groups. Thus, additional resonances at δ **3.75** and **3.73** (additional to the presumed aliphatic methoxy-group resonance present at δ **3.66**), which arise from two aromatic methoxy-groups, occur in the ^{1}H n.m.r. spectrum ($CDCl_3$) of compound (1b), which is obtained upon methylation of compound (1a) with diazomethane, and reveal the presence of two phenolic hydroxy-groups in the parent compound. Acetylation of compound (1b) gives the dimethyl ether triacetate (1c) which exhibits proton resonances due to three aliphatic acetoxy-groups (δ **1.84**, **1.91**, and **2.00**), while two two-proton multiplets at δ **2.47** and **4.06**, due to the benzylic and carbohydrate methylene moieties, respectively, are distinguishable. The ^{1}H n.m.r. spectrum of neoraufuracin penta-acetate (1d) differs from that of compound (1c) by the presence of two acetoxy-resonances in the place of the two aromatic methoxy-resonances (*cf.* Experimental section).

In order to elucidate the structure, the penta-acetate (1d), selected as the most suitable of these derivatives, was subjected to high-resolution (500 MHz) ^{1}H n.m.r. spectroscopy in different solvents ($CDCl_3$ and C_6D_6) at high temperatures (310—320 K). The relative differences in the chemical shifts at this frequency enable the complete assignment of the resonances and elucidation of the structure by decoupling experiments.

Together with the aromatic ABX and AA_1BB_1 systems two independent four- and seven-proton spin systems [corresponding to the partial structures (3) and (4)] are



(3)



(4)

identified. From the n.m.r. data it is evident that the aglycone portion comprises two aromatic rings linked by a central C_3H_4 unit. This four-spin heterocyclic system is identified by the resonances at δ 4.97br (d, $J_{2.3}$ 9.25 Hz), 3.87br (t), and 2.79 and 2.56 (dd × 2, C_6D_6) attributed to 3- and 2-H, and the benzylic β-methylene protons, respectively. The benzylic character of 3-H, as indicated by the broadening of the signal by long range coupling, is confirmed by the sharpening in the doublet signal of 4-H upon irradiation of 3-H, which also transforms the triplet of 2-H, broadened by dynamic rotational isomerism, into a broad doublet ($J_{2.3}$ *ca.* 9.25 Hz). Irradiation of 2-H simplifies the signal of 3-H to a broad singlet, while each of the β-methylene protons resonates as a doublet ($J_{\beta,\beta'}$ 14.5 Hz). Proof of the benzylic nature of the last-mentioned protons is provided by the sharpening in the signals of both upon irradiation of 2′- and 6′-H. In the gated decoupled 20.1 MHz ^{13}C n.m.r. spectrum of neoraufuracin (1a) C-2 and C-3 appear as doublets (J 140 Hz) at δ_C 74.6 and 81.8 p.p.m., respectively, while C-β resonates as a triplet (J 125 Hz) at δ_C 36.4 p.p.m., which confirms the above assignments. The identity of the aglycone moiety is confirmed by hydrolysis of compound (1a) with hydriodic acid which yields the hydroxybenzofuran (14a), characterized as the dimethyl ether (14b) by t.l.c. comparison with a synthetic sample.

The carbohydrate moiety is defined by the resonances of the seven-spin system. Irradiation of the signal of 1″-H resolves the doublet of doublets due to 2″-H into a doublet ($J_{2'',3''}$ 10.0 Hz), while decoupling of 2″-H similarly simplifies the doublet of doublets attributable to 3″-H to a doublet ($J_{3'',4''}$ 9.25 Hz) and the doublet representing 1″-H to a singlet. Decoupling of 3″-H causes 4″-H and 2″-H to appear as doublets ($J_{4'',5''}$ 10.0 Hz, and $J_{1'',2''}$ 7.75 Hz, respectively), while the signals of 6″a-H, 6″b-H, and 4″-H each change, upon irradiation of 5″-H, from a doublet of doublets ($J_{6''a,6''b}$ 12.5 Hz) to a doublet. The cited coupling constants illustrate [3] the overall axial orientation of these protons and thus identify the carbohydrate residue as β-D-glucopyranose. The β-anomeric configuration is further confirmed by the resonance of the anomeric carbon atom at δ_C 107 p.p.m. in the ^{13}C n.m.r. spectrum of compound (1a) in contrast to the resonance at δ_C *ca.* 99 p.p.m. of α-anomeric carbon atoms.[4]

The chemical shift of the carbon of the methoxy-group in the ^{13}C n.m.r. spectrum of neoraufuracin (1a) (δ_C 54.7 p.p.m.) is in line with those of methyl-β-D-glucopyranose derivatives (δ_C 54.7—56.7 p.p.m.) rather than of their 2″-methyl ethers (δ_C 57.5—60.7 p.p.m.),[4,5] thus defining its position at C-1″ in a methyl-β-D-glucopyranosyl unit. Similarly, its ^1J[^{13}C–H(1″)] coupling (150 Hz) also, apparently, confirms its β-orientation when compared with similar anomeric couplings of α- and β-methyl-glucopyranosides (170 and 160 Hz respectively).[6]

The downfield resonances of 3″-H and 4″-H in compound (1d) indicate the location of two of the three heterocyclic acetoxy-groups. The change in chemical shift of 6″a-H and 6″b-H upon acetylation ($\Delta\delta$ *ca.* 0.3) is expected for a hydroxymethyl function, and since 2″-H resonates at relatively high field and experiences little change upon acetylation, this proton is placed geminal to the 3-*O*-(2-benzyl-2,3-dihydrobenzofuranyl) group. The presence of this ether function may explain the resistance of neoraufuracin (1a) to enzymic hydrolysis.

Rationalisation of the foregoing leads to the assignment of structure (1a), 6-hydroxy-2-(4-hydroxybenzyl)-3-*O*-(methyl-β-D-glucopyranos-2-yl)-*trans*-2,3-dihydrobenzofuran, to neoraufuracin, since no alternative which satisfies these requirements could be formulated.

The 500 MHz ^{1}H n.m.r. spectrum of ambofuracin tetra-acetate (2b) is a replica of that of neoraufuracin penta-acetate (1d) except for an additional three proton *O*-methyl singlet (δ 3.35), an additional coupled four-

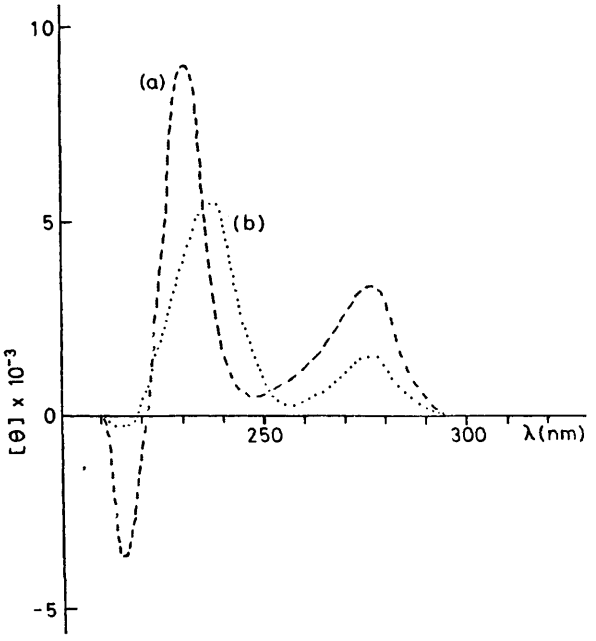


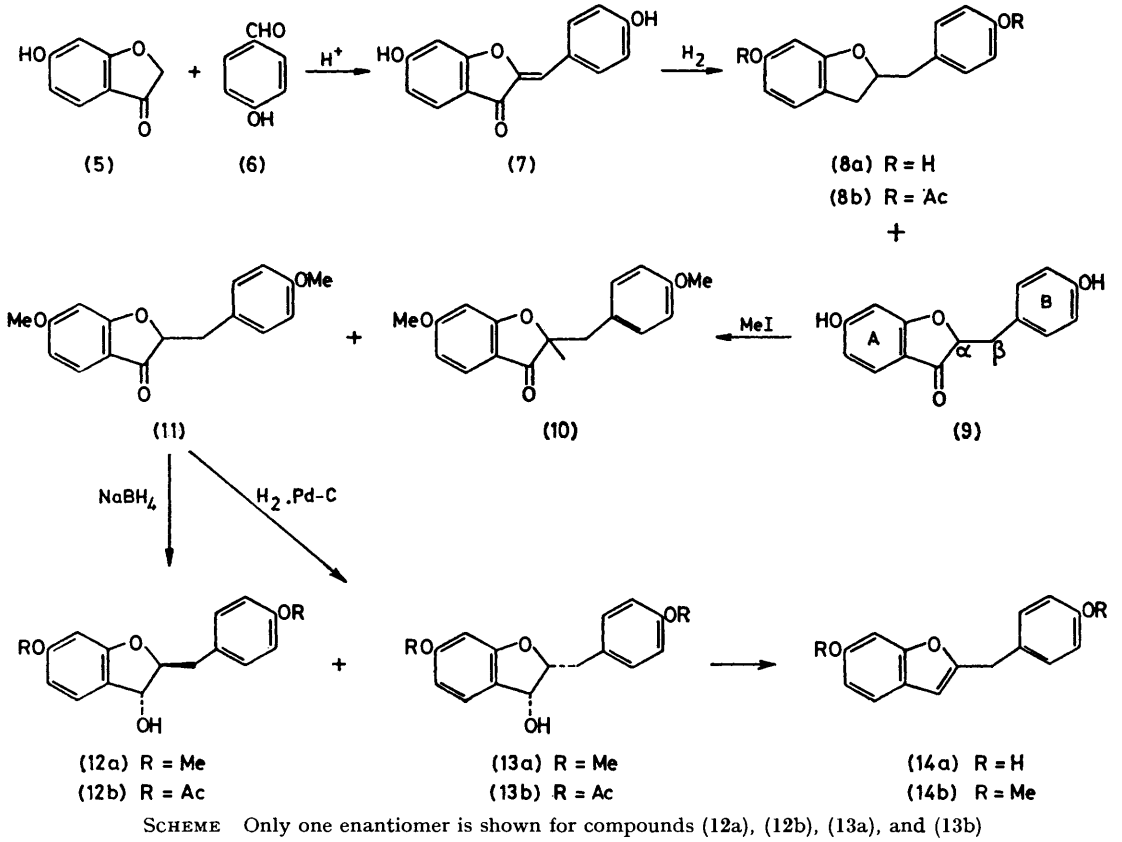
FIGURE C.d. spectra of (a) neoraufuracin (- - - -) and (b) ambofuracin (· · · · ·)

proton multiplet (δ 2.42—2.20),* and the absence of one acetoxy-group signal. An identical full decoupling experiment identified the aglycone also as a 3-oxygenated 2-benzyl-4′,6-dihydroxy-2,3-*trans*-dihydrobenzofuran and the hexose residue as methyl-β-D-glucopyranose. The only discernible relative difference in chemical shifts is that of the methylene function at C-6″, which shows that the differing substitution is confined to this position (*cf.* Experimental section). Decoupling also indicates that the four-proton multiplet forms an independent spin system. The methyl ester character of the additional methoxy-group singlet is implied by its low field resonance (δ 3.35), while the narrow deshielded multiplet (δ 2.42—2.20) formed by two methylene moieties indicates their vicinal position and near magnetic equivalence. The indication that the additional 6″-functionality represents an esterified methylsuccinyl derivative of the methyl-β-D-glucopyranosyl moiety is confirmed by the molecular ion at *m/e* 716 in the field-desorption mass spectrum of ambofuracin (2a), which corresponds to the expected molecular formula of $C_{27}H_{34}O_{12}$.

Stereochemically, the compounds (1a) and (2a) are identical; both exhibit positive Cotton effects at λ 230—240 nm and at λ 270—280 nm (Figure). The coupling constant $J_{2,3}$ 9.25 Hz indicates a 2,3-*trans* relative stereochemistry, which is supported by ^{1}H n.m.r. spectral data of the synthetic aglycone (see later). Since no analogous structures of known stereochemistry could be traced,† the absolute configuration of these compounds could not be assigned.

Partial confirmation of the structures of neoraufuracin (1a) and ambofuracin (2a) was attempted by the synthesis of a derivative of the aglycone portion of (1a). Acid-catalysed condensation [8] of 6-hydroxybenzofuran-3-one (5) and *p*-hydroxybenzaldehyde (6) yields the aurone hispidol (7) almost quantitatively. Catalytic hydrogenation of hispidol (7) under drastic conditions provided tetrahydrohispidol (8a) and α,β-dihydrohispidol (9) (Scheme).

α,β-Dihydrohispidol (9) resists hydride reduction



SCHEME Only one enantiomer is shown for compounds (12a), (12b), (13a), and (13b)

(NaBH$_4$ or LiAlH$_4$), but yields, upon methylation with methyl iodide, the desired hispinidol (11) as the major product, as well as 2-methylhispinidol (10), as was expected from the acidity of the α-hydrogen atom. Hispinidol (11) gives the two racemates (12a) and (13a) upon hydride reduction followed by column chromatography on silica gel. The identity of the *cis*-isomer (13a), which exhibits $J_{2,3}$ 3.0 Hz in contrast to the greater coupling ($J_{2,3}$ 5.0 Hz) of the *trans*-isomer, is confirmed

* This occurs as a singlet at 80 MHz.

† The o.r.d. data of benzofuranoid neolignans (ref. 7) do not provide sufficiently close analogies. However, the positive high intensity Cotton effects at low wavelengths (λ 230—240 nm) indicate 3*S* and hence 2*R*,3*S* absolute configurations, in terms of the aromatic quadrant rule, are likely for both 2,3-*trans* compounds (1a) and (2a).

by catalytic hydrogenation which gives the *cis*-isomer as the only product. The magnitudes of ^{1}H n.m.r. coupling constants of other *cis*- and *trans*-2,3-dihydrobenzofurans do not provide information as regards the relative stereochemistry of these compounds, since all three possible correlations have been reported: J_{cis} 5—7 Hz > J_{trans} 2—4 Hz;[9] J_{cis} 3—4 Hz < J_{trans} 5—6 Hz;[10] and $J_{cis} \approx J_{trans}$ 7—9 Hz.[11] The large coupling constant (J 9 Hz) of the natural products (1) and (2) agrees with results obtained from other natural dihydrobenzofurans.[11]

Attempts to condense the benzylic alcohols (12a) or (13a) regiospecifically with the 2-hydroxy-function of methyl-β-D-glucopyranose were not made. Dehydration occurs easily, especially with the *cis*-compound (13a), which gives the 2-benzylbenzofuran (14).

Neoraufuracin (1a) and ambofuracin (2a) may originate biosynthetically from the aurone hispidol (7) through a reduction sequence similar to that outlined in the synthetic scheme followed by methylglucosylation and further functionalisation. Notable, however, is the conspicuous absence of aurones or chalcones from *N. amboensis* and the low concentrations of flavonoids in contrast to the over-abundance of isoflavonoids.

Neoraufuracin and ambofuracin are unusual in that, as far as we are aware, they represent the first reported examples of methyl-β-D-glucopyranose units ether-linked to a benzylic position of a flavonoid.

EXPERIMENTAL

M.p.s were determined with a Reichert Thermopan microscope. I.r. spectra were recorded for chloroform solutions on a Unicam SP-1000 spectrophotometer. Optical rotations were measured on a Bendix NP-2 polarimeter for solutions in chloroform, while c.d. curves were determined on a JASCO J-20 apparatus for solutions in methanol. Mass spectra were recorded on a Varian CH-5 double-focusing mass spectrometer, while field-desorption mass spectra were recorded by Dr. U. Rapp, Varian MAT GmbH, Bremen. ^{1}H (80 MHz) and ^{13}C (20.1 MHz) N.m.r. spectra were recorded on a Bruker WP-80 FT instrument using tetramethylsilane as the internal standard and $CDCl_3$ as the solvent, unless otherwise specified. Values of J (^{1}H–^{13}C) are derived from gated decoupling experiments. ^{1}H N.m.r. (500 MHz) spectra were recorded on a Bruker WM-500 FT spectrometer.

Merck silica gel 60 was used for column chromatography and Merck silica gel PF_{254} for preparative t.l.c. Unless otherwise specified R_F values refer to chromatography on precoated Merck t.l.c. plastic sheets (silica gel 60 F_{254}), and colour reactions to perchloric acid–iron(III) chloride spray reagent. Qualitative two-dimensional paper chromatography was done on Whatman No. 1 paper using 2% acetic acid and water saturated butan-2-ol in sequence.

Isolation of Compounds from the Root Bark of N. amboensis. —Bulbs of *N. amboensis* were collected near Tshokwane, Kruger National Park, Transvaal. The bark was removed, dried, and powdered to give 910 g of material which was successively extracted with hexane (3 × 24 h), benzene (3 × 24 h), and methanol (3 × 24 h) in a Soxhlet apparatus, and produced a brown semi-solid (67 g, 7.4%), a brown amorphous solid (93 g, 10.2%), and a black syrup (456 g, 50.1%), respectively.

A portion (180 g) of the methanol extract was dissolved in the lower phase of a saturated ethyl acetate–water mixture, diluted to 200 cm^3, and fed into the first 8 tubes of a Steady State Distribution Apparatus (Quickfit Instruments, Model 20), programmed for 103 transfers with an agitation time of 5 min and a settling time of 90 min. The contents of the tubes were combined into five fractions, as shown in the Table, following two-dimensional paper

Upper phase		Fraction number	Lower phase	
Tube Nos.	Mass (g)		Tube Nos.	Mass (g)
1—10	0.24	1	1—11	165.2
11—33	0.55	2	12—35	3.35
34—74	1.27	3	36—75	0.71
75—98	2.10	4	76—98	0.15
99—103	6.14	5	99—103	0.03

chromatography. The procedure was repeated with a second portion (200 g) of the extract. Fractions 4 and 5 of the upper phase contained known isoflavonoids, while fraction 1 of the lower phase consisted of sucrose (*ca.* 10%), D-glucose, amino-acids, and five unidentified isoflavone glycosides. The combined fractions 3 (upper and lower phases) were purified by column chromatography [chloroform–ethanol (9 : 1, v/v)] and t.l.c. [benzene–acetone–methanol (16 : 4 : 1 v/v/v)] to give neoraufuracin (1a) (251 mg) and ambofuracin (2a) (10 mg).

Neoraufuracin (1a) fluoresced weakly (λ 254 nm) to give purple, R_F 0.18 [benzene–methanol (4 : 1)], 0.36 [chloroform–ethanol (17 : 3)], and 0.98, 0.67 (Whatman No. 1) and yellow-brown with bis-diazotised benzidine, red-brown with diazotised sulphanilic acid, yellow with diazotised 4-nitroaniline, and red with Millon's reagent. Neoraufuracin (1a) was obtained as *needles* (methanol), m.p. 228—230 °C; $\nu_{max.}$ (KBr) 1 130, 1 220, 1 310, 1 530, 1 630, 1 890, 1 950, 3 000, and 3 300—3 500 cm^{-1}; $[\alpha]_D^{20}$ −125.0° (*c* 0.20); c.d. (*c* 0.12) $[\theta]_{210}$ 0, $[\theta]_{215}$ −3 074, $[\theta]_{220}$ 0, $[\theta]_{230}$ +9 041, $[\theta]_{246}$ +543, $[\theta]_{275}$ +3 436, and $[\theta]_{295}$ 0 (Found: C, 60.4; H, 5.8%; M^+, 434. $C_{22}H_{26}O_9$ requires C, 60.8; H, 6.0%; *M*, 434); δ_H (80 MHz, [$^2H_6$]acetone) 7.22 (d, *J* 8.75 Hz, 4-H), 6.81 (d, *J* 8.75 Hz, 2′- and 6′-H), 6.59 (d, *J* 8.75 Hz, 3′- and 5′-H), 6.47 (d, *J* 2.5 Hz, 7-H), 6.41 (dd, *J* 8.75 and 2.5 Hz, 5-H), 4.69 (d, *J* 9 Hz, 3-H), 4.38 (d, *J* 7.75 Hz, 1″-H), 3.75 (s, OMe), 3.06 (dd, *J* 10 and 7.75 Hz, 2″-H), and *ca.* 2.50 (m, CH_2); δ_C ([$^2H_5$]pyridine) 36.4 (t, *J* 125 Hz, CH_2Ph), 54.7 (q, *J* 137.5 Hz, 1″-OMe), 61.9 (t, *J* 137.5 Hz, C-6″), 70.9 (d, *J* 145 Hz, C-4″), 74.3 (d, *J* 145 Hz, C-2″), 74.6 (d, *J* 140 Hz, C-2), 80.0 and 80.2 (2 d, *J* 145 Hz, C-3″ and -5″), 81.8 (d, *J* 140 Hz, C-3), 98.4 (dd, *J* 155 and 5 Hz, C-5), 98.6 (dd, *J* 155 and 5 Hz, C-7), 107.3 (d, *J* 150 Hz, C-1″), 114.7 (dd, *J* 155 and 5 Hz, C-3′ and -5′), 116.3 (d, *J* 5 Hz, C-3a), 127.5 (d, *J* 5 Hz, C-1′), 128.4 (d, *J* 155 Hz, C-4), 129.4 (dd, *J* 155 and 5 Hz, C-2′ and -6′), 155.2 (d, *J* 5 Hz, C-4′), 156.7 (d, *J* 5 Hz, C-7a), and 158.3 p.p.m. (d, *J* 5 Hz, C-6).

4′,7-Di-*O*-methylneoraufuracin (1b) gave purple, R_F 0.30 (chloroform); δ_H 7.22 (d, *J* 8.75 Hz, 4-H), 6.88 (d, *J* 8.75 Hz, 2′- and 6′-H), 6.56 (d, *J* 8.75 Hz, 3′- and 5′-H), 6.37 (dd, *J* 8.75 and 2.5 Hz, 5-H), 6.27 (d, *J* 2.5 Hz, 7-H), 4.68 (d, *J* 9 Hz, 3-H), 4.31 (d, *J* 7.8 Hz, 1″-H), 3.75, 3.73, and 3.66 (3 s, 3 × OMe), 2.50 (dd, *J* 10 and 7.8 Hz, 2″-H), *ca.* 1.88 (m, β-CH_2), and 4.00—3.15 (m, all other protons).

3″,4″,6″-Tri-*O*-acetyl-4′,7-di-*O*-methylneoraufuracin (1c) gave purple, R_F 0.33 [chloroform–hexane (4 : 1)]; δ_H 7.09 (d, *J* 8.75 Hz, 4-H), 6.84 (d, *J* 8.75 Hz, 2′- and 6′-H), 6.56 (d, *J* 8.75 Hz, 3′- and 5′-H), 6.34 (dd, *J* 8.75 and 2.5 Hz, 5-H), 6.25 (d, *J* 2.50 Hz, 7-H), 5.09 (t, *J* 10 Hz, 4″-H), 4.91

(t, J 10 Hz, 3''-H), 4.66 (d, J 9 Hz, 3-H), 4.34 (d, J 7.8 Hz, 1''-H), 4.06 (t, 6''-H), 3.69 (2 s, 2 × OMe), 3.63 (s, OMe), 3.66 (dd, J 10 and 7.8 Hz, 2''-H), 2.47 (m, β-CH_2), and 2.00, 1.91, and 1.84 (3 s, 3 × OAc).

Neoraufuracin penta-acetate (1d) gave purple, R_F 0.24 [chloroform–hexane (4 : 1)] and was obtained as white needles (acetone), m.p. 202—203 °C; δ_H (500 MHz, $CDCl_3$) 7.37 (d, J 8.50 Hz, 4-H), 7.04 (dd, J 8.75 and 2.25 Hz, 2'- and 6'-H), 6.90 (dd, J 8.75 and 2.25 Hz, 3'- and 5'-H), 6.72 (dd, J 8.50 and 2.25 Hz, 5-H), 5.24 (dd, J 10.00 and 9.25 Hz, 4''-H), 5.07 (dd, J 10.00 and 9.25 Hz, 3''-H), 4.85br (d, J 9.00 Hz, 3-H), 4.48 (d, J 7.75 Hz, 1''-H), 4.24 (dd, J 12.50 and 5.00 Hz, 6''a-H), 4.14 (dd, J 12.50 and 2.25 Hz, 6''b-H), *ca.* 3.82 (m, 2- and 5''-H), 3.80 (s, OMe), 3.48 (dd, J 10.00 and 7.75 Hz, 2''-H), 2.77 (dd, J 15.00 and 9.50 Hz, β-H), 2.52 (dd, J 15.00 and 2.50 Hz, β'-H), and 2.28, 2.23, 2.06, 1.99, and 1.95 (5 s, 5 × OAc); δ_H (500 MHz, C_6D_6) 7.42 (d, J 8.50 Hz, 4-H), 6.98 (dd, J 8.75 and 2.25 Hz, 2'- and 6'-H), 6.92 (dd, J 8.75 and 2.25 Hz, 3'- and 5'-H), 6.77 (dd, J 8.50 and 2.25 Hz, 5-H), 6.51 (d, J 2.25 Hz, 7-H), 4.34 (dd, J 12.50 and 4.50 Hz, 6''a-H), 4.13 (dd, J 12.50 and 2.25 Hz, 6''b-H), 3.57 (octet, J 10.00, 4.50, and 2.25 Hz, 5''-H), 5.48 (dd, J 10.00 and 9.25 Hz, 4''-H), 5.35 (dd, J 10.00 and 9.25 Hz, 3''-H), 3.55 (dd, J 10.00 and 7.75 Hz, 2''-H), 4.21 (d, J 7.75 Hz, 1''-H), 4.97br (d, J 9.25 Hz, 3-H), 3.87br (t, 2-H), 2.79 (dd, J 14.50 and 9.00 Hz, β-H), 2.56 (dd, J 14.50 and 2.50 Hz, β'-H), and 1.80, 1.67, 1.66, 1.65, and 1.56 (5 s, 5 × OAc).

Ambofuracin (2a) developed purple, R_F 0.82 [chloroform–ethanol (17 : 3)] and was obtained as a white amorphous solid, c.d. (*c* 0.01) $[\theta]_{210}$ 0, $[\theta]_{215}$ −510, $[\theta]_{219}$ 0, $[\theta]_{243}$ +5 100, $[\theta]_{258}$ +481, $[\theta]_{281}$ +1 470, and $[\theta]_{295}$ 0; δ_H ([$^2H_6$]acetone) 7.15 (d, J 8.75 Hz, 4-H), 6.78 (d, J 8.75 Hz, 2'- and 6'-H), 6.56 (d, J 8.75 Hz, 3'- and 5'-H), 6.41 (d, J 2.5 Hz, 7-H), 6.34 (dd, J 8.75 and 2.5 Hz, 5-H), 4.63 (d, J 8 Hz, 3-H), 4.34 (d, J 7.75 Hz, 1''-H), 3.78 and 3.56 (2 s, 2 × OMe), 2.8—2.2 (m, 3 × CH_2), and 3.5—4.5 (m, all other protons); *m/e* 716 (field desorption).

Ambofuracin tetra-acetate (2b) gave purple, R_F 0.32 (chloroform) and was obtained as needles (acetone), m.p. 197—198 °C; δ_H (500 MHz, C_6D_6) 7.42 (d, J 8.5 Hz, 4-H), 6.96 (dd, J 8.7 and 2.3 Hz, 2'- and 6'-H), 6.90 (dd, J 8.7 and 2.3 Hz, 3'- and 5'-H), 6.78 (dd, J 8.5 and 2.3 Hz, 5-H), 6.49 (d, J 2.3 Hz, 7-H), 5.50 (dd, J 10.0 and 9.25 Hz, 4''-H), 5.34 (dd, J 10.0 and 9.25 Hz, 3''-H), 5.00br (d, J 9.25 Hz, 3-H), 4.35 (dd, J 12.5 and 4.5 Hz, 6''a-H), 4.20 (d, J 7.75 Hz, 1''-H), 4.18 (dd, J 12.5 and 2.25 Hz, 6''b-H), 3.83br (s, 2-H), 3.58 (m, 2''- and 5''-H), 3.35 (s, CO_2Me), 3.11 (s, 2'-OMe), 2.78 (dd, J 14.5 and 9.0 Hz, β-H), 2.58 (dd, J 14.5 and 2.5 Hz, β'-H), 2.42—2.20 (m, $COCH_2CH_2CO$), and 1.80, 1.73, 1.71, and 1.55 (4 s, 4 × OAc).

Hydrolysis with Hydriodic Acid.—Neoraufuracin (1a) (10 mg) and phenol (60 mg), dissolved in hydriodic acid (1 cm^3), was stirred at room temperature for 20 min after which $NaHSO_3$ (2 cm^3, 37%) was added. The mixture was extracted with ethyl acetate, washed, dried (Na_2CO_3), and evaporated to dryness. Methylation of the residue yielded 6-methoxy-2-(4-methoxybenzyl)benzofuran (14b), identified by t.l.c. comparison with an authentic sample.

Synthesis of the 4',6-*Di*-O-*methylneoraufuracidins* (12a) *and* (13a)

(Z)-*Hispidol* (7).[8]—*p*-Hydroxybenzaldehyde (6) (12.2 g, 0.01 mol) in ethanol (30 cm^3) was mixed with 6-hydroxy-2*H*-1-benzofuran-3-one (5) (14.0 g, 0.1 mol) in ethanol (40 cm^3) and heated to 60 °C. Hydrochloric acid (2.5 cm^3, conc.) was added and the mixture was stirred until it solidified (*ca.* 90 min), whereupon it was filtered and the residue crystallised (ethanol) to yield (*Z*)-hispidol (7) (19.1 g, 75.2%) as orange needles, m.p. 285—290 °C (decomp.) (lit.,[12] 287—290 °C, decomp.).

Tetrahydrohispidol (8a) *and* α,β-*Dihydrohispidol* (9).[8]—Hydrogenation of (*Z*)-hispidol (7) (5 g) in ethanol (70 cm^3) for 6 h at 60 °C and 10 atm with 10% Pd–C, filtration, and column chromatography [chloroform–acetone (4 : 1)] yielded two products.

(i) Tetrahydrohispidol (8a) (1.30 g, 27.3%) as white needles (acetone), m.p. 171—172 °C, R_F 0.61, purple, *m/e* 242 (28.8%, M^+), 238 (5.1), 148 (29.5), 135 (76.9), 134 (59.3), and 107 (100); δ_H ([$^2H_6$]acetone) 7.94br (s, OH), 6.97 (d, J 8.75 Hz, 2'- and 6'-H), 6.78 (d, J 8.75 Hz, 4-H), 6.62 (d, J 8.75 Hz, 3'- and 5'-H), 6.16 (dd, J 8.75 and 2.5 Hz, 5-H), 6.09 (d, J 2.5 Hz, 7-H), 4.84 (m, 2-H), and 3.19—2.63 (m, 3-H and CH_2); and 4',6-di-*O*-acetyltetrahydrohispidol (8b) as white *plates* (carbon tetrachloride), m.p. 121—122 °C (Found: C, 69.8; H, 5.5. $C_{19}H_{18}O_5$ requires C, 69.9; H, 5.6%); *m/e* 326 (2.2%, M^+); δ_H 7.09 (d, J 8.75 Hz, 2'- and 6'-H), 6.91 (d, J 8.75 Hz, 4-H), 6.84 (d, J 8.75 Hz, 3'- and 5'-H), 6.41 (dd, J 8.75 and 2.5 Hz, 5-H), 6.34 (d, J 2.5 Hz, 7-H), 4.91 (m, 2-H), 3.28—2.66 (m, 3-H and CH_2), and 2.19 and 2.22 (2 s, 2 × OAc).

(ii) α,β-Dihydrohispidol (9) (2.74 g, 54.4%) as white *needles* (chloroform), m.p. 165—166 °C, orange, R_F 0.52 (Found: C, 70.1; H, 4.6. $C_{15}H_{12}O_4$ requires C, 70.3; H, 4.7%); *m/e* 256 (20.6%, M^+); δ_H ([$^2H_6$]acetone) 7.28 (d, J 8.75 Hz, 4-H), 7.00 (d, J 8.75 Hz, 2'- and 6'-H), 6.59 (d, J 8.75 Hz, 3'- and 5'-H), 6.44 (dd, J 8.75 and 2.5 Hz, 5-H), 6.34 (d, J 2.5 Hz, 7-H), 4.69 (dd, J 7 and 4 Hz, 2-H), 3.18 (dd, J 15.0 and 4.0 Hz, β-H), and 2.81 (dd, J 15.0 and 7.0 Hz, β'-H).

Hispinidol (11) *and* 2-*Methylhispinidol* (10).—Methylation of α,β-dihydrohispidol (9) (1.8 g) with methyl iodide and subsequent chromatography [chloroform–acetone (4 : 1)] gave two products.

(i) 2-Methylhispinidol (10) (205 mg, 9.78%) as *glass*, R_F 0.50, brown (Found: M^+, 298.1194. $C_{18}H_{18}O_4$ requires M, 298.1205); *m/e* 298 (5.9%, M^+); δ_H 7.31 (d, J 8.75 Hz, 4-H), 7.00 (d, J 8.75 Hz, 2'- and 6'-H), 6.56 (d, J 8.75 Hz, 3'- and 5'-H), 6.38 (dd, J 8.75 and 2.5 Hz, 5-H), 6.31 (d, J 2.5 Hz, 7-H), 3.75 and 3.66 (2 s, 2 × OMe), 2.94 (s, β-H), and 1.38 (s, Me).

(ii) Hispinidol (11) (1.33 g, 66.6%) as *needles* (ethanol), m.p. 90—91 °C, R_F 0.49, yellow (Found: C, 71.8; H, 5.7. $C_{17}H_{16}O_4$ requires C, 71.8; H, 5.7%); *m/e* 284 (20.7%, M^+); δ_H 7.37 (d, J 8.75 Hz, 4-H), 7.06 (d, J 8.75 Hz, 2'- and 6'-H), 6.66 (d, J 8.75 Hz, 3'- and 5'-H), 6.44 (dd, J 8.75 and 2.5 Hz, 5-H), 6.34 (d, J 2.5 Hz, 7-H), 4.63 (dd, J 7.0 and 4.0 Hz, 2-H), 3.75 and 3.69 (2 s, 2 × OMe), 3.25 (dd, J 15.0 and 4.0 Hz, β-H), and 2.81 (dd, J 15.0 and 7.0 Hz, β'-H).

trans- *and* cis-4',6-*Di*-O-*methylneoraufuracidin* (12a) *and* (13a).—To hispinidol (11) (150 mg) in tetrahydrofuran (0.5 cm^3) was added $NaBH_4$ (100 mg) in tetrahydrofuran (0.5 cm^3) and the mixture was stirred overnight at room temperature. Acetone (1 cm^3) was added and the solvent was evaporated in a stream of air at room temperature. Column chromatography [chloroform–acetone (19 : 1)] of the residue gave three products.

(i) *trans*-4',6-Di-*O*-methylneoraufuracidin (12a) (70 mg, 46.3%) as white *needles* (chloroform), m.p. 105—106 °C, R_F 0.52, purple (Found: C, 71.3; H, 6.4. $C_{17}H_{18}O_4$ requires C, 71.3; H, 6.3%); *m/e* 286 (16.9%, M^+); δ_H

7.13 (m, 4-H), 7.16 (d, J 8.75 Hz, 2′- and 6′-H), 6.72 (d, J 8.75 Hz, 3′- and 5′-H), 6.34 (dd, J 8.75 and 2.5 Hz, 5-H), 6.25 (d, J 2.5 Hz, 7-H), 4.81 (t, J 8.5 Hz, 3-H), 4.50 (m, 2-H), 3.68 and 3.63 (2 s, 2 × OMe), 3.06 (octet, β-CH_2), and 1.72 (d, J 7.5 Hz, OH); δ_H ($CDCl_3$ + D_2O) 4.81 (d, J 5.0 Hz, 3-H).

(ii) *cis*-4′,6-Di-*O*-methylneoraufuracidin (13a) (33.1 mg, 21.8%) as *glass*, R_F 0.41, purple (Found: M^+, 286.1211. $C_{17}H_{18}O_4$ requires M, 286.1205); *m/e* 286 (6.3%, M^+); δ_H 7.09 (m, 4-H), 7.03 (d, J 8.75 Hz, 2′- and 6′-H), 6.72 (d, J 8.75 Hz, 3′, and 5′-H), 6.46 (dd, J 8.75 and 2.5 Hz, 5-H), 6.28 (d, J 2.5 Hz, 7-H), 4.90 (m, 3-H), 4.63 (m, J 7.5 and 2.5 Hz, 2-H), 3.75 and 3.73 (2 s, 2 × OMe), 2.81 (octet, CH_2), and 1.81 (d, J 7.5 Hz, OH); δ_H ($CDCl_3$ + D_2O) 4.90 (d, J 3.0 Hz, 3-H).

(iii) 6-Methoxy-2-(4-methoxybenzyl)benzofuran (14b) as white *needles* (ethanol), m.p. 108—109 °C, purple, R_F 0.85 (Found: C, 76.1; H, 5.9. $C_{17}H_{16}O_3$ requires C, 76.1; H, 6.0%); *m/e* 268 (100%, M^+); δ_H 7.19 (d, J 8.75 Hz, 4-H), 7.03 (d, J 8.75 Hz, 2′- and 6′-H), 6.68 (m, 3′-, 5′-, 5- and 7-H), 6.13 (dd, J 1.0 and 0.5 Hz, 3-H), 3.9br (s, CH_2), and 3.75 and 3.71 (2 s, 2 × OMe).

We thank Mr. P. van Wyk, Skukuza, Kruger National Park, Transvaal for collection and Dr. M. E. Oberholzer for extraction of the plant material; Dr. U. Rapp, Varian MAT GmbH, Bremen for field-desorption mass spectra; Drs. J. M. Steyn and E. V. Brandt for electron-impact mass spectra and 80 MHz ^1H and 20.1 MHz ^{13}C n.m.r. spectra, respectively; and the Council for Scientific and Industrial Research, Pretoria and the Sentrale Navorsingsfonds, University of the Orange Free State, for financial support.

[1/1313 *Received*, 17*th August*, 1981]

REFERENCES

[1] J. C. Breytenbach and G. J. H. Rall, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1804.
[2] M. E. Oberholzer, G. J. H. Rall, and D. G. Roux, *Phytochemistry*, 1976, **15**, 1283.
[3] R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, 1958, **80**, 6098.
[4] E. Breitmaier and W. Voelter, '^{13}C-N.m.r. Spectroscopy,' Verlag Chemie, Berlin, 1974, 223.
[5] M. Miljkovic, M. Gligorijevic, T. Satoh, D. Glisin, and R. G. Pitcher, *J. Org. Chem.*, 1974, **39**, 3847.
[6] K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, 1974, 293.
[7] O. R. Gottlieb, J. C. Mourao, M. Yoshida, Y. P. Mascarenhas, M. Rodrigues, R. D. Rosenstein, and K. Tomita, *Phytochemistry*, 1977, **16**, 1003.
[8] J. S. Hastings and H. G. Heller, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2128.
[9] M. P. Mertes, L. J. Powers, and E. Sefter, *J. Org. Chem.*, 1971, **36**, 1805; J. Srogl, M. Janda, I. Stibor, and R. Rozinek, *Synthesis*, 1975, 717; C. H. Ludwig, B. J. Nist, and J. L. McCarthy, *J. Am. Chem. Soc.*, 1964, **86**, 1186.
[10] L. H. Zalkow and M. Ghosal, *J. Org. Chem.*, 1969, **34**, 1646.
[11] M. Gregson, W. D. Ollis, B. T. Redman, I. O. Sutherland, and H. H. Dietrichs, *Chem. Commun.*, 1968, 1394; C. A. Anirudhan, D. W. Mathieson, and W. B. Whalley, *J. Chem. Soc. C*, 1966, 634.
[12] E. Wong, *Phytochemistry*, 1966, **5**, 463.