Pergamon

0031-9422(94)00824-8

Phytochemistry, Vol. 38, No. 6, pp. 1505–1508, 1995
Elsevier Science Ltd
Printed in Great Britain
0031-9422/95 \$9.50 + 0.00

ISO-OCOBULLENONE AND A NEOLIGNAN KETONE FROM *OCOTEA BULLATA* BARK

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(*Received* 10 *August* 1994)

Key Word Index—*Ocotea bullata*; Lauraceae; bark; *iso*-ocobullenone; neolignan ketone.

Abstract—A new neolignan, which differs from ocobullenone at a single chiral centre, and a neolignan ketone, possibly a precursor of the ocobullenones, have been isolated from the stem bark of *Ocotea bullata*.

INTRODUCTION

We have previously reported on the isolation of ocobullenone (**1**) from *Ocotea bullata*. It is a bicyclo[3.2.1]octanoid neolignan, possessing the less-common 7.1^1, 8.3^1 coupling between units [1]. Determination of its structure was based on sophisticated NMR studies (HMBC and ROESY) and it was possible to draw certain conclusions regarding relative stereochemistry (as depicted in **1**) from NOE measurements. Ocobullenone, together with the two new compounds reported in this paper, constitute the major components found in the bark of *O. bullata* and may well represent the active agents in the medicinal 'cocktail' present in this endangered natural resource. Compounds of the neolignan-type have so far not been found in other Lauraceae (for example, *Cryptocarya latifolia*) occurring in southern Africa [2]. The 'chemical link' between *Ocotea* and *Cryptocarya* species still remains unsubstantiated at this stage, despite the practice of traditional healers of substituting *Cryptocarya* extractives for the rapidly diminishing reserves of *Ocotea* bark.

RESULTS AND DISCUSSION

For the sake of clarity this discussion will deal first with *iso*-ocobullenone (**2**) and thereafter with the neolignan ketone **3**. Comparison of the ^{1}H NMR spectra in chloroform-*d* of ocobullenone and *iso*-ocobullenone reveals immediate differences in the chemical shift of the protons attached to C-7 and C-8. The remaining protons show relatively little change in chemical shift (Table 1).

By comparing the proton and carbon spectra of *iso*-ocobullenone (**2**) with ocobullenone (**1**) (both in $CDCl_3$) it could be established that they were probably stereoisomers. This view was further reinforced by examining the same spectral data for **2** in benzene-d_6 as solvent. In this solvent, there was less overlap of peaks and a definite assignment of all carbon and proton atoms was possible.

High resolution mass spectrometry confirmed that **1** and **2** were indeed stereoisomers with a molecular formula of $C_{21}H_{22}O_6$. Since the optical rotations of the two compounds were both positive, but not identical, it was reasonable to assume that they were diastereomers and it remained to establish where the configurational differences lay. In this regard, the CD spectra of **1** and **2** provided us with helpful information; the two curves were practically superimposable, except for a small, but distinct difference in absorption at 226 nm.

Final confirmation of the structure of *iso*-ocobullenone was obtained from X-ray analysis of suitable crystals (Fig. 1). Study of the X-ray structure indicates that ocobullenone (**1**) and *iso*-ocobullenone (**2**) have identical structures and that the configurations at three of the four stereocentres are identical. It is in fact at C-8 that they have opposite configurations. In ocobullenone (**1**), the C-9 methyl group, the benzene ring and the cyclohexenone ring all lie above the general plane of the cyclopentane ring. In *iso*-ocobullenone (**2**), the methyl group is below the plane of the cyclopentane ring. At this stage, it is not possible to assign absolute configurations at the chiral centres. The X-ray diagram as well as a Dreiding model of **2** indicate that C-7, C-8, C-1^1 and C-3^1 are effectively coplanar with C-2^1 buckled out of plane. The same is true for cyclohexenone ring which adopts a five-point coplanar configuration with C-2^1 once more out-of-plane.

The third compound to be isolated in low concentration from *O. bullata* stem bark was a neolignan ketone, for which the structure **3** is proposed. It was isolated after repeated chromatographic separations of the ethyl acetate extract and exists as a crystalline solid, mp 103°. Its

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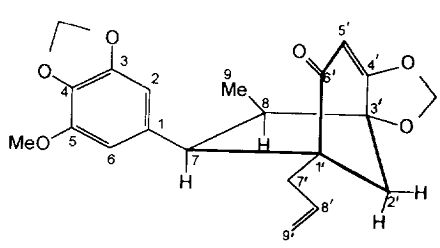

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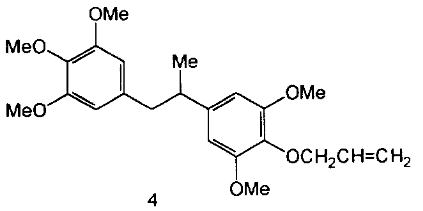

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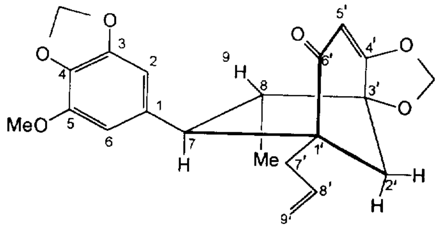

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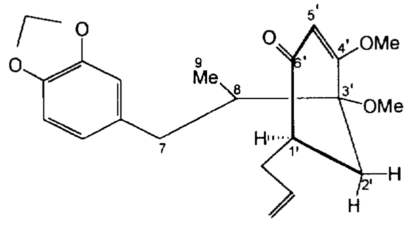

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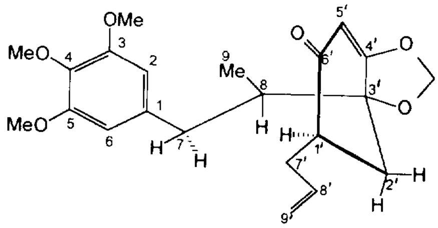

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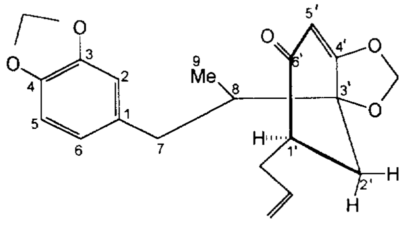

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Table 1. ^{1}H NMR spectral data of compounds **1** (in $CDCl_3$, 500 MHz) and **2** (in $CDCl_3$ and C_6D_6, 200 MHz) (δ in ppm from TMS, J in Hz)

		2	
H	**1**	$CDCl_3$	C_6D_6
2, 6	6.12 *dd* (1.70)	6.24, 6.26 *dd* (1.65)	6.35, 6.40 *dd* (1.63)
3,4-methylenedioxy	5.87, 5.89 *dd* (1.52)	5.90, 5.92 *dd* (1.44)	5.23, 5.29 *dd* (1.44)
5-OMe	3.93 *s*	3.86 *s*	3.53 *s*
7	3.37 *d* (11.90)	2.68 *d* (5.88)	2.45 *d* (6.04)
8	2.87 *dq* (11.90, 7.40)	2.48 *dq* (13.7)	2.25 *m*
9-Me	0.85 *d* (7.42)	1.16 *d* (6.90)	0.90 *d* (6.94)
2^1a	2.06 and 2.26	2.08 *dd* (10.81, 1.28)	1.87 and 2.05
2^1b	*dd* (10.50)	and 2.34 *d* (10.81	*dd* (10.71)
3^14^1-methylenedioxy	5.63, 5.67 *dd* (0.33)	5.46, 5.73 *dd* (0.32)	4.62, 4.96 *dd* (0.33)
5^1	5.57 *s*	5.43 *s*	5.48 s
7^1a	2.05 *dd* (14.18, 8.94)	2.07 *dd* (14.20, 8.88)	1.92 *m*
7^1b	2.56 *dddd* (14.11, 5.75, 1.39)	2.67 *dddd* (13.94, 5.80, 1.29)	2.87 *dddd* (13.90, 5.50, 1.30)
8^1	5.75 *m*	5.66–5.85 *m*	5.71–5.90 *m*
9^1	5.06 *m*	5.07–5.18 *m*	4.97–5.08 *m*

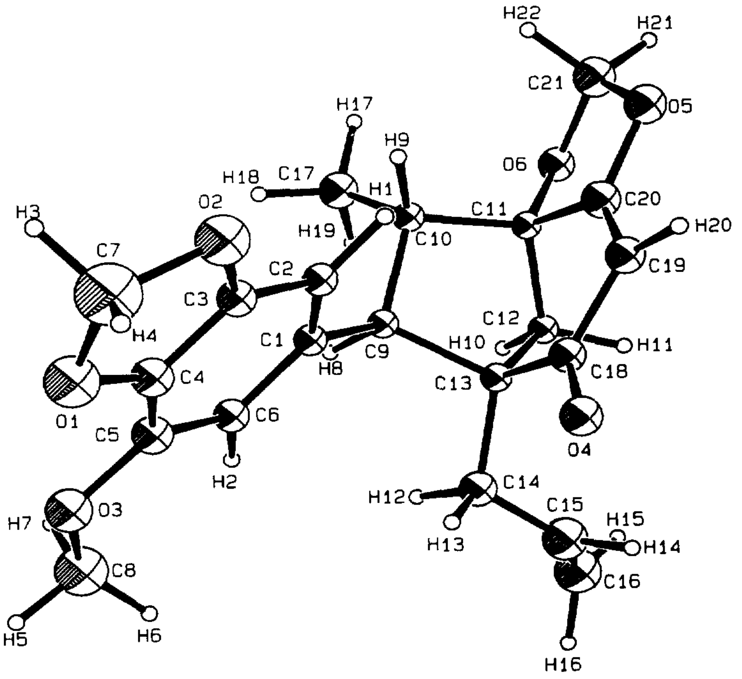

Fig. 1. X-ray structure of compound **2**. Note orientation of methyl group on C-10.

structure was established using standard ^1H–^{13}C techniques coupled with COSY, HETCOR and HMBC analysis at 500 MHz. The relationship of **3** to **1** is apparent. In **3**, there is an intact 8,3^1-linkage, but the 7,1^1 bond is severed at the carbon α to the carbonyl group at C-6^1. Mass spectrometry of **3** showed a $[M]^+$ of *m/z* 388.1865, which corresponds to a molecular formula of $C_{22}H_{28}O_6$. The presence of three chemically equivalent sp^2 carbons was readily explained by the presence of a 3,4,5-trimethoxybenzene moiety. A similar situation exists in the neolignan aurein, **4** [3]. Spectral information available for isodihydrofutoquinone **5**, isolated by Matsui [4], and for $\Delta 8^1$-3^1,6^1-dihydro-3,4,3^1,4^1-*bis*-methylenedioxy-6^1-oxo-8,3^1 neolignan (**6**), obtained by Green and Wiener [5], proved to be very helpful for purposes of comparison. The allyl side-chain on C-1^1 was discernible from both the ^1H and ^{13}C spectra; the chemical shifts were comparable to those observed in ocobullenone. The methylenedioxy group at C-3^1, C-4^1 was characterized by the typical methylene carbon shift at δ99.9 and the corresponding protons at δ5.60. The carbons of the α-β unsaturated system had chemical shifts very similar to those found in ocobullenone. The 8,3^1-linkage was established on the basis of connectivities discernible from an HMBC experiment (Table 2).

The planarity constraints of the α,β-unsaturated system, coupled with the requirements of the methylenedioxy group, lend substantial rigidity to the cyclohexenone ring resulting in a pseudo-chair conformation in which carbons 1^1, 6^1, 5^1, 4^1 and 3^1 exhibit five-point coplanarity. This results in the aryl/alkyl unit being attached to an axial bond (at C-3^1) of the cyclohexenone ring. NOE experiments also show a strong correlation between H-1^1 and H-8, as well as between H-1^1 and H-9 (Me). The above information then places the allyl side-chain (at C-1^1) on an equatorial bond and in a *trans* orientation relative to the aryl/alkyl moiety attached to C-3^1. The new neolignan ketone **3** has an obvious close structural relationship to both ocobullenone (**1**) and *iso*-ocobullenone (**2**) and may well act as precursor for one or the other.

Table 2. HMBC correlations to establish linkage between the two ring systems in compound **3**

H	Correlated carbon
1^1	6^1, 8^1, 7^1
2^1a	6^1, 4^1, 3^1, 1^1
2^1b	3^1, 8, 1^1, 7^1
5^1	6^1, 4^1, 3^1, 1^1
7a	3, 1, 2, 3^1, 8 9
7b	1, 2, 9
7^1a, 7^1b	6^1, 8^1, 9^1, 1^1, 2^1
8	1, 3^1, 7, 9
9	3^1, 8, 7
9^1	8^1, 7^1
OMe-4	4
OMe-3,5	3, 5
O-CH_2-O	3^1, 4^1

EXPERIMENTAL

Mps: uncorr. ^1H and ^{13}C NMR spectra were recorded at 200, 500 and 50, 125 Hz, respectively; $CDCl_3$ or C_6D_6

were used as solvents. Plant material was collected in January 1994 from mature trees of *O. bullata* growing in the Karkloof range, district of Pietermaritzburg. Identification was made by R.S.-S. and a voucher specimen is deposited in the CPF Herbarium, Queen Elizabeth Park, Pietermaritzburg.

Extraction and isolation. Dry, finely milled bark (3 kg) was extracted successively with hexane, $CHCl_3$ and EtOAc. From the $CHCl_3$ extract (45 g), a portion (11.4 g) was repeatedly fractionated (MPLC) on silica gel 60 using Et_2O–petrol (3:7) to give *iso*-ocobullenone (18 mg). It was very difficult to separate ocobullenone (the dominant component) and *iso*-ocobullenone since they have almost identical R_fs on TLC in a variety of solvent systems. Thus, in one of the best systems, Et_2O–hexane (3:7), the R_fs are 0.39 and 0.33 for (**1**) and (**2**) respectively (repeat run); compound **3** has an R_f of 0.19.

Iso-*ocobullenone* (**2**). Needles, mp 142–144°. $[\alpha]_D^{23}$ + 122.8° ($CHCl_3$; *c* 0021). ^{1}H NMR in $CDCl_3$ and C_6D_6 (Table 1). ^{13}C NMR: δ 16.9 (C-9), 37.1 (C-7^1), 43.2 (C-2^1), 43.6 (C-8), 56.7 (OMe), 58.2 (C-1^1), 59.5 (C-7), 87.4 (C-3^1), 97.0 (C-5^1), 99.3 (aliph OCH_2O–), 101.4 (arom., –OCH_2O–), 102.2 (C-6), 108.7 (C-2), 118.5 (C-9^1), 132.2 (C-1), 134.5 (C-8^1), 134.5 (C-4), 142.9 (C-5), 148.8 (C-3), 179.1 (C-4^1), 198.7 (C-6^1). EI-MS *m/z* (rel. int.): 370 $[M]^+$ (31), 325 (24), 270 (13), 219 (24), 178 (100), 137 (49), 115 (24). HR-MS calcd for $C_{21}H_{22}O_6$: 370.1415, found 370.1400.

X-Ray analysis. Iso-ocobullenone recrystallized from CH_2Cl_2–hexane as orthorhombic crystals, cell parameters $a = 7.036$ (12), $b = 9.186$ (3), $c = 28.768$ (9) Å, space group $P2_12_12_1$ $Z = 4$. The diffraction intensities were collected on a CAD-4 diffractometer using monochromated MoK_α radiation. The structure was solved by direct methods and the final R values were 0.1040 for 701 reflections. The configuration shown in Fig. 1 is based on an arbitrary choice of parameters.

Extraction and isolation of compound **3**. From the EtOAc extract, a brown powder (9.1 g) was obtained and fractionated using MPLC and $CHCl_3$ initially, followed by gradient elution using petrol–EtOAc. This afforded **3**, $\Delta 8^1$-3,4,5-trimethoxy-3^1,6^1-dihydro-3^1,4^1-methylenedioxy-6-oxo-8,3^1-neolignan (14 mg) as needles, mp 105°. $[\alpha]_D^{25}$ + 175° (CH_2Cl_2; *c* 0.08). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.99 (3H, *d*, $J = 6.7$ Hz, Me-9), 1.78 (1H, *dd*, $J = 12.7$ Hz, H-2^{1}b), 2.09 (1H, *ddq*, $J = 12.6$, 6.8, 2.6 Hz, H-8), 2.25 (1H, *dd*, $J = 12.6$, 11.8 Hz, H-7b), 2.26 (1H, *m*, H-7^{1}b), 2.40 (1H, *m*, H-1^1), 2.56 (1H, *dd*, $J = 5.20$ Hz, H-2^{1}a), 2.69 (1H, *m*, H-7^{1}a), 2.87 (1H, *dd*, $J = 12.6$, 2.4 Hz, H-7a), 3.80 (3H, *s*, OMe-4), 3.82 (6H, *s*, OMe H-3^1, H-5^1), 5.07 (2H, *m*, H-9^1), 5.55 (1H, *s*, H-5^1), 5.60 (2H, *dd*, $J = 7.9$ Hz, –OCH_2–O), 5.70 (1H, *m*, H-8^1), 6.27 (2H, *s*, H-2, H-6). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.3 (C-9), 35.7 (C-2^1), 36.3 (C-7^1), 38.8 (C-7), 41.2 (C-8), 42.4 (C-1^1), 56.2 (OMe-3, OMe-5), 60.8 (OMe-4), 85.4 (C-3^1), 99.9 (C-5^1), 100.0 (–OCH_2O–), 106.1 (C-2, C-6), 117.6 (C-9^1), 135.2 (C-8^1), 135.6 (C-1), 136.8 (C-4), 153.2 (C-3, C-5), 175.8 (C-4^1), 198.8 (C-6^1). EI-MS *m/z* (rel. int.): 388 $[M]^+$ (8), 209 (35), 208 (42), 193 (16), 182 (18), 181 (100), 148 (19), 139 (48), 137 (20). HR-MS calcd for $C_{22}H_{28}O_6$: 388.1886, found 388.1865.

Acknowledgements—The authors thank the Foundation for Research Development (FRD) and the University Research Fund for financial assistance. Prof. D Ferreira (Bloemfontein) is sincerely thanked for recording CD spectra.

REFERENCES

1. Sehlapelo, B. M., Drewes, S. E. and Sandor, P. (1993) *Phytochemistry* **32**, 1352.
2. Drewes, S. E., Sehlapelo, B. M., Horn, M. and Scott-Shaw, R. (1994) *Phytochemistry* **37**, 847.
3. Wenkert, E., Gottlieb, H. E., Gottlieb, O. R., Perreira, M. O. and Formiga, M. D. (1976) *Phytochemistry* **15**, 1547.
4. Matsui, K. (1976) *Tetrahedron Letters* 4371.
5. Green, T. P. and Wiener, D. F. (1991) *Phytochemistry* **30**, 3759.