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ISOLATION AND STRUCTURE ELUCIDATION OF XYLOBUXIN, A NEW NEOLIGNAN FROM *XYLOPIA BUXIFOLIA*

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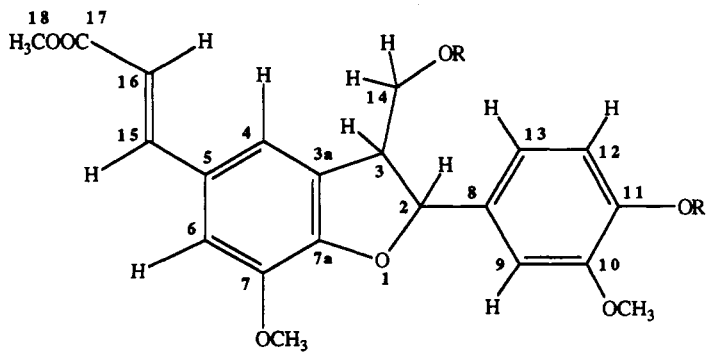
ABSTRACT.—The structure of a new lignan named xylobuxin, isolated from the stem bark of *Xylopia buxifolia*, has been elucidated by spectroscopic methods, mainly 1D and 2D nmr, and chemical transformations. The relative stereochemistry for xylobuxin was deduced by nOe spectroscopy and calculations from models.

The stem bark of *Xylopia buxifolia* Baill. (Annonaceae) (1) has been re-investigated for its chemical constituents. Besides the known isoquinoline alkaloids (2–6), methyl 3,4-dimethoxybenzoate and a new lignan [**1**], named xylobuxin, were isolated. This paper deals with the structural elucidation of this new compound on the basis of spectroscopic and chemical evidence.

A CH_2Cl_2-soluble fraction of the aqueous MeOH extract afforded xylobuxin [**1**] as an amorphous white powder. Compound **1** showed a M^+ at *m/z* 386 (eims), which is consistent with the formula $C_{21}H_{22}O_7$. The ir spectrum revealed an absorption band at 1725 cm^{-1}, indicative of an ester group, and this was confirmed by ^{13}C-nmr data (a carbonyl carbon signal at δ 168.2 ppm). The ^1H-nmr spectrum (Table 1) revealed the presence of five aromatic protons enclosed in two aromatic systems: an AX system corresponding to a 1,2,3,5-tetrasubstituted ring and an AMX system corresponding to a 1,2,4-trisubstituted ring. In the same area of the ^1H-nmr spectrum the typical pattern of a trans ethylenic system was observed at δ 6.15 (d, J=16 Hz) and 7.45 (d, J=16 Hz). In addition, three signals at δ 3.71, 3.44, and 5.44 were attributed to an aliphatic CH-CH-CH_2 link, and three MeO groups were observed at δ 3.63, 3.67, and 3.75. The presence of two OH groups was shown by diacetylation of **1**. At this stage, the sequence of the carbons could not be determined because of the large number of quaternary carbons.

The analysis of the HMBC data (Table 1) provided more information about the two aromatic systems. The protons of the MeO group at δ 3.63 (C-18) showed a ^3J correlation with the carbonyl group at δ 168.2 (C-17), which had correlations with the ethylenic protons at δ 6.15 and 7.47



1 R=H
3 R=$COCH_3$

TABLE 1. ^{13}C- (50 MHz) and ^{1}H- (200 MHz) Nmr Data ($CDCl_3$, δ) for Xylobuxin [**1**].

Position	δ_C	δ_H (*J*, Hz)	NOe observations[a]	HMBC[a] (C to H)
2	88.6 (d)	5.44 (d, *J*=6 Hz)	H-3, H-9, H-13, H-14	H-3, H-9, H-13, H-14
3	53.0 (d)	3.44 (td, *J*=6 and 6 Hz)	H-9	H-2, H-4, H-14
3a	129.2 (s)			H-3, H-14
4	117.6 (d)	6.96 (br s)	H-14, H-15, H-16	H-6, H-15
5	127.9 (s)			H-16
6	111.7 (d)	6.86 (br s)	H-15, H-16, OCH_3-7	H-4, H-15
7	144.2 (s)			H-6, OCH_3-7
7a	150.3 (s)			H-2, H-3, H-4, H-6
8	132.2 (s)			H-2, H-3, H-9, H-12
9	109.1 (d)	6.75 (d, *J*=1.7 Hz)	H-2, OCH_3-10	H-2, H-13
10	147.3 (s)		H-9	H-9, H-12, OCH_3-10
11	145.9 (s)			H-9, H-12, H-13
12	114.7 (d)	6.65 (d, *J*=8 Hz)		
13	118.7 (d)	6.69 (dd, *J*=1.7 and 8 Hz)	H-2	H-2, H-9
14	63.2 (t)	3.71 (d, *J*=6 Hz)	H-2, H-4	H-2, H-3
15	145.3 (d)	7.47 (d, *J*=16 Hz)	H-4, H-6	H-4, H-6
16	114.3 (d)	6.15 (d, *J*=16 Hz)	H-4, H-6	H-15
17	168.2 (s)			H-15, H-16, OCH_3-17
OCH_3-7	55.6 (q)	3.75 (s)	H-6	
OCH_3-10 ...	55.4 (q)	3.67 (s)	H-9	
OCH_3-17 ...	51.2 (q)	3.63 (s)		

[a]NOe difference and HMBC experiments were conducted at 400 MHz.

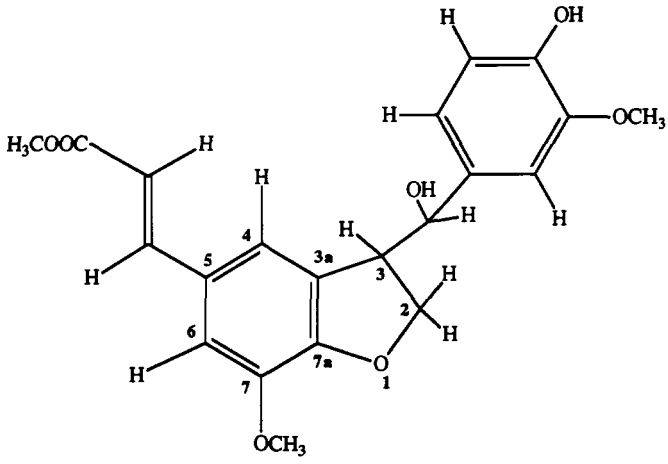
(C-15 and 16). Moreover, the ethylenic proton at δ 6.15 had a ^{3}J correlation with the quaternary carbon at δ 127.9 (C-5), and the ethylenic proton at δ 7.47 showed ^{3}J correlations with both aromatic carbons at δ 111.7 (δ_H 6.86) (C-6) and 117.6 ppm (δ_H 6.96) (C-4).

In addition, the nOe between the aromatic proton at δ 6.86 (H-6), and the methoxy protons at δ 3.75 [bonded to the aromatic carbon at δ 144.2 (C-7)] revealed the position of the latter group at C-7 on the cinnamate moiety. The ^{3}J correlations of the other oxygenated carbon (δ 150.3) (C-7a) with both aromatic protons at δ 6.86 and 6.96 indicated the presence of an alkoxy group at C-7a.

The second aromatic system was trisubstituted by an alkyl group at C-8 (δ_C 132.2) and two oxygenated substituents at C-10 and C-11 as shown by the ^{1}H-nmr coupling constants. The two protons at δ 6.69 (H-13) and 6.75 (H-9) were correlated (^{3}J) with the OH-bearing carbon at δ 145.9, indicating its position at C-11. The other oxygenated carbon at δ 147.3 (C-10) was linked to the MeO group (δ_H 3.67) and correlated with the third aromatic proton (δ_H 6.65), thus indicating the C-10 position for the MeO functionality.

At this point, knowing that the aliphatic moiety CH-CH-CH_2 was bonded to the adjacent carbons at δ 129.2 (C-3a) and 150.3 (C-7a) of the first aromatic ring, and the carbon at δ 132.2 (C-8) of the second aromatic ring, 16 structures were possible. After a careful analysis of the HMBC spectrum, only two structures were retained, xylobuxin [**1**] and the alternative structure [**2**].

Several observations suggested structure **1** for xylobuxin. The resonance of the geminal protons at δ 3.71 as one doublet (*J*=6 Hz) indicating the magnetic equivalence of the two protons was in favor of a primary alcohol and excluded the presence of a cyclic methylene. The observation of a nOe (Table 1) between the methylene at δ 3.71 and the aromatic proton at δ 6.96 (H-4) also excluded structure **2** and favored structure **1**. Finally, compound **1** was peracetylated by $(Ac)_2O$ in pyridine and afforded the diacetylated derivative [**3**]. The downfield shift of the methylene group (from δ 3.71 to 4.25) and of the α-methine group (from δ 3.44 to 4.30), as well as the



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unchanged chemical shift of the methine at δ 5.44, were further proof of the presence of the primary alcohol.

The relative configuration of the protons at C-2 and C-3 has been determined by comparison of nmr and molecular modeling studies. The calculated value for a trans configuration was $J=10.5$ Hz. The coupling constant observed in **1** between the protons at C-2 and C-3 was $J=6$ Hz, corresponding to a cis configuration.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The optical rotation was measured on a Perkin-Elmer 241 polarimeter. The uv spectrum was registered on a Philips PU 8720 spectrophotometer and the ir spectrum on a Perkin-Elmer 841. Eims data were collected on a Kratos MS-80 spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AC200P instrument and 2D homonuclear and heteronuclear correlated nmr spectra on a Bruker ARX 400 instrument.

PLANT MATERIAL.—The stem bark of *Xylopia buxifolia* Baill. was collected in Ampanatoamaizina, Madagascar, in March 1986, and identified at the herbarium of the ORSTOM center at Antananarivo, Madagascar, where a voucher specimen (No. P. 704) has been deposited.

EXTRACTION AND ISOLATION.—Powdered stem bark (2 kg) was extracted by percolation with MeOH (40 liters). The MeOH extract was concentrated under reduced pressure, diluted with H_2O, and extracted sequentially with hexane and CH_2Cl_2. The aqueous layer, alkalinized with 25% NH_4OH, was extracted with CH_2Cl_2 to afford an extract containing the alkaloids and certain neutral compounds. This extract was subjected to chromatography on Si gel using CH_2Cl_2 with increasing percentages of MeOH (up to 50%) as a gradient eluent. Further purifications were carried out using prep. tlc over Si gel. Methyl 3,4-dimethoxybenzoate (15 mg) was obtained using CH_2Cl_2 as eluent, and 25 mg of **1** purified using the solvent mixture hexane-EtOAc (20:80).

Xylobuxin [**1**].—$[\alpha]^{20}D$ +7° (c=0.3, MeOH); uv λ max (log ε) (MeOH) 205 (4.38), 224 (4.12), 329 (4.06) nm; ir ($CHCl_3$) ν max 3550, 3165, 2945, 1725 cm^{-1}; eims *m/z* 386 (M^+, 63), 368 ($M-H_2O$, 100), 356 (39), 353 (33), 238 (44), 167 (65), 137 (75); ^{1}H-, ^{13}C-, and HMBC nmr data, see Table 1.

Xylobuxin diacetate [**3**].—A solution of xylobuxin [**1**] (15 mg) in pyridine (0.5 ml) was treated with $(Ac)_2O$ (1 ml) and stirred at room temperature for 24 h. The reaction mixture was then hydrolyzed and extracted three times with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated to dryness under reduced pressure, affording a diacetylated compound [**3**] in 95% yield after prep. tlc purification on Si gel using CH_2Cl_2-MeOH (95:5) as eluent; eims *m/z* 470 (M^+, 31), 428 ($M-CH_2CO$, 4), 410 ($M-CH_2CO-H_2O$, 5), 368 ($M-2(CH_2CO)-H_2O$, 100); ^{1}H nmr δ 2.00 (3H, s $OCOCH_3$-14), 2.25 (3H, s, $OCOCH_3$-11), 3.76, 3.80, and 3.90 (3×3H, 3×s, 3×OCH_3), 4.25 (2H, d, J=6 Hz, CH_2-14), 4.30 (1H, td, J=6 and 6 Hz, H-3), 5.52 (1H, d, J=6 Hz, H-2), 6.21 (1H, d, J=16 Hz, H-16), 6.79 (5H, m, aromatic H), 7.51 (1H, d, J=16 Hz, H-15).

ACKNOWLEDGMENTS

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