# LIGNANS OF PIPER CUBEBA\*

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Key Word Index—*Piper cubeba*; Piperaceae; diarylcyclobutane neolignans; dibenzylbutyrolactone lignan; *O*-ethyl cubebins; monoacetate of dihydrocubebin; structural determination.

Abstract—Seven additional compounds have been isolated from *Piper cubeba* and characterized as heterotropan, magnosalin, 2,4,5-trimethoxybenzaldehyde,  $\alpha$ - and  $\beta$ -O-ethyl cubebins, 5"-methoxybinokinin and the monoacetate of dihydrocubebin. The latter four compounds are new natural products.

## INTRODUCTION

The isolation and characterization of (-)-cubebin, (-)hinokinin, (-)-clusin, (-)-cubebinin, (-)-dihydrocubebin and (-)-dihydroclusin [1] as well as (-)yatein, (-)-cubebininolide (cordigerine), (-)-2-(3",4"methylenedioxybenzyl)-3-(3',4'-dimethoxybenzyl)butyrolactone, (-)-isoyatein, (-)-cubebinone and (-)-di-Omethyl thujaplicatin methyl ether [2] from the fruits of *Piper cubeba* have already been described.

In continuation of the phytochemical investigation of the same source, we now wish to report the isolation of seven more compounds, of which four are new lignans.

### **RESULTS AND DISCUSSION**

Compounds 1 and 2 were identified as two isomeric diarylcyclobutane type neolignans, viz. magnosalin (1) [3] and heterotropan (2) [4]. Acoradin [5] and bisasarin [6] are two more examples of such lignans. Compound 3 was found to be 2,4,5,-trimethoxybenzaldehyde [5]. The remaining four compounds, which were new natural products, were characterized as  $\alpha$ -O-ethyl cubebin (4),  $\beta$ -O-ethyl cubebin (5), 5"-methoxyhinokinin (6) and the monoacetate of dihydrocubebin (hemiariensin) (7).

\* Part 3 in the series "Lignans from Piper cubeba". For Part 2, see ref. [2].

Compounds 4, 5 and cubebin (8) showed a close resemblance with one another since they furnished pink coloured spots when the TLC plate was heated at  $100^{\circ}$  after spraying with H<sub>2</sub>SO<sub>4</sub> (10%). Subsequently compounds 4 and 5 were separated by multiple prep. TLC.

Compounds 4 and 5, both viscous masses, were found to be isomers,  $C_{22}H_{24}O_6$ , ([M]<sup>+</sup> m/z 384),  $R_f$  0.59 and 0.63, respectively, (C<sub>6</sub>H<sub>6</sub>-EtOAc, 49:1, twice developed). Their IR spectra indicated the presence of aromaticity, an ether linkage and methylenedioxy groups. Besides, both showed a typical lignanoid type of UV absorption pattern (234 and 287 nm). The presence of an ethoxy group in each compound was evident from the three proton triplets at  $\delta 1.18$  and  $\delta 1.21$ , respectively, in their <sup>1</sup>H NMR spectra as well as the appearance of peaks at m/z 338 [M  $-C_2H_3OH^{\dagger}$  in their mass spectra. The orientation of the ethoxy group was, however, established by comparing the signals for the C-2 protons with those of  $\alpha$ - and  $\beta$ methyl cubebins (9 and 10) [7] in their <sup>1</sup>H NMR spectra (see Table 1). Moreover, these two compounds were found to be identical with the two isomeric products obtained by the base catalysed reaction of ethyl bromide with cubebin (8, of known 3R,4R configuration) in all respects (co-TLC, CD, IR, <sup>1</sup>H NMR). Consequently, the structures of compounds 4 and 5, i.e.  $\alpha$ - and  $\beta$ -O-ethyl cubebins, were elucidated to be [(2S,3R, 4R)-3,4-bis-(3',4'-methylenedioxybenzyl)]- $2\alpha$ -ethoxy-furan and [(2R, 3R, 4R)-3,4-bis- $(3', 4'-methylenedioxybenzyl]-2\beta$ -ethoxy-furan, respectively. Although ethoxyclusin (11) is known to occur in P.

Table 1. <sup>1</sup>H NMR values ( $\delta$ , 100 MHz, CDCl<sub>3</sub>) of compounds 4, 5, 9 and 10

Compound - OCH <sub>2</sub> CH <sub>3</sub>		H-3, H-4, H-6, H-7	-OC <u>H</u> 3	H-5	ОС <u>Н</u> ₂СН,	Н-2	-осн, о-	Ar–H
4	1.18 (3H, t,	2.0-2.86		3.28-4.08 (4H, m)		4.83 ( <i>β</i> H,	5.95, 5.97	6.46-6.84
	J = 6 Hz)	(6H, m)				d, J = 2 Hz)	(4H, s each)	(6H, m)
9	_	2.0-2.82	3.29	4.01 (1H, $dd$ , $J_1 =$	_	4.71 (βH,	5.92 (4H, s)	6.42-6.80
		(6H, m)	(3H, s)	7 Hz, $J_2 = 9$ Hz)		d, J = 1.2  Hz		(6H, m)
5	1.21 (3H, t,	1.80-2.84	_	3.22-4.10 (4H, m)		4.78 (aH,	5.96 (4H, s)	6.62-6.90
	J = 7  Hz	(6H, m)				d, J = 4  Hz		(6H, <i>m</i> )
10	—	1.80-2.96	3.34	4.01 (1H, $t, J = 8$	-	4.68 (aH,	5.94 (4H, s)	6.62-6.90
		(6H, m)	(3H, s)	Hz), 3.58 (1H, dd,		d, J = 4.5  Hz		
				$J_1 = 6$ Hz, $J_2 = 8$ Hz)				



Ar = 2,4,5 - trimethoxyphenyl -









8 R + R<sup>1</sup> = OCH<sub>2</sub>O, R<sup>2</sup> = R<sup>3</sup> = H 11 R = R<sup>1</sup> = R<sup>2</sup> = OMe, R<sup>3</sup> = C<sub>2</sub>H<sub>5</sub>



 $\mathbf{R} = \mathbf{R}^1 = \mathbf{A}\mathbf{c}$ 

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clusii [8], this forms the first report of the occurrence of  $\alpha$ and  $\beta$ -O-ethyl cubebins.

Compound 6, viscous mass,  $C_{21}H_{20}O_7$  ([M]<sup>+</sup> m/z 384), closely resembled hinokinin (12) in its UV and IR spectra. Its <sup>1</sup>HNMR spectra suggested that this lignan, too, belonged to the 2,3-trans-dibenzylbutyrolactone group. The comparison of the molecular formulae of 6 and 12 indicated that the latter compound contained an additional methoxy group. The location of this group was determined with the help of its <sup>1</sup>H NMR and mass spectra. The appearance of fragment ion peaks at m/z 165 and 135 revealed the presence of a methoxy-methylenedioxybenzyl group and a methylenedioxybenzyl moiety, respectively. That the former group was attached to C-2 of the  $\gamma$ lactone was confirmed by the presence of peaks at m/z 222 and 249 in accordance with such observations made earlier [2]. Its <sup>1</sup>H NMR displayed a singlet at  $\delta 6.33$  which was attributed to a meta-related H-2" and H-6" of the benzylic group attached to C-2 [2]. Consequently, a two proton multiplet at  $\delta 6.49$  was assigned to H-2' and H-6' and a doublet at  $\delta 6.72 (J = 8 \text{ Hz})$  to H-5'. Both, hinokinin and 6 in their CD spectra exhibited negative Cotton effects in the region of 236 and 286 nm confirming an identical (2R, 3R) configuration. Therefore, the structure of compound **6** was elucidated to be (2R, 3R)-2-(5''-methoxy-3'', 4''-methylenedioxybenzyl)-3-(3', 4'-methylenedioxybenzyl)-butyrolactone (6). It was named 5''-methoxyhinokinin.

Compound 7, viscous mass,  $C_{22}H_{24}O_7$  ([M]<sup>+</sup> m/z 400), showed many spectral features in common with dihydrocubebin (13) [1] and ariensin (14) [10] suggesting that the compound could be a derivative of a 2,3-dibenzylbutanediol lignan. The comparison of molecular formulae of all the three compounds indicated that 7 was a monoacetoxy derivative of 13. This was further supported by its mass spectral fragmentation pattern (m/z 340, [M - AcOH]<sup>+</sup>) and <sup>1</sup>H NMR spectral data (see Experimental). Finally, 7 was easily acetylated to yield a derivative which was identical in all respects (co-TLC, IR and NMR) with the diacetate of dihydrocubebin, i.e. ariensin (14) which has been reported from *Bursera ariensis* [10]. Therefore, compound 7, 4-acetoxy-2,3-bis-(3,4-methylenedioxybenzyl)-butan-1-ol, was named as hemiariensin.

It is interesting to postulate the biogenesis of 7. It is



Fig. 1. Possible biogenesis of hemiariensin.

known that a dibenzylbutyrolactol lignan exists as an equilibrium mixture of two tautomeric forms, viz. cyclic lactol and its corresponding open-chain 4-hydroxy aldehyde (Fig. 1). The isolation of hemiariensin (7) suggested that the acetylation of the 4-hydroxy aldehyde form preceded the reduction of the aldehydic group.

#### **EXPERIMENTAL**

<sup>1</sup>H NMR (100 MHz): CDCl<sub>3</sub>. MS (70 eV) direct insertion.  $[\alpha]_{D}^{25}$ : CHCl<sub>3</sub>. UV: MeOH. CD: MeOH. IR: CHCl<sub>3</sub>. Chromatographic separations were carried out on silica gel or alumina (Brockmann grade II). TLC was performed on silica gel G using C<sub>6</sub>H<sub>6</sub>-EtOAc. Spots were detected in UV (254 nm), exposing the plates to I<sub>2</sub> vapour and heating to 100° in an oven after spraying with H<sub>2</sub>SO<sub>4</sub> (10%).

Extraction and isolation of compounds. The general procedure for isolation of compounds has been described earlier [2]. Compounds 4 and 5 were obtained from the less polar fractions which eluted prior to cubebin. The more polar fractions, together with the mother liquor obtained after the crystallization of cubebin, were chromatographed successively over silica gel and alumina. The individual compounds were, however, obtained in pure form by repeated prep. TLC on silica gel.

Magnosalin (1) and 2,4,5-trimethoxybenzaldehyde (3) were identified by comparing their physical and spectral data with those in the lit. [3, 5]. Heterotropan (2) was identified by direct comparison with an authentic sample (co-TLC, IR, NMR) kindly supplied by Prof. M. Niwa.

α-O-Ethylcubebin [(2S, 3R, 4R)-3,4-bis-(3,4-methylenedioxybenzyl)]-2α-ethoxy-furan (4). Viscous mass,  $R_f$  0.59 (C<sub>6</sub>H<sub>6</sub>-EtOAc, 49:1 twice developed), UV  $\lambda_{max}^{MeOH}$  nm (log ε): 234 (3.93) and 287 (3.89). IR v cm<sup>-1</sup>: 2970, 2900, 1610, 1500, 1450, 1250, 1040, 920 and 870. CD:  $\Delta \epsilon_{230}$  -0.86 and  $\Delta \epsilon_{290}$  -0.67 (MeOH; c 0.06). <sup>1</sup>H NMR: see Table 1. MS m/z (rel. int): 384 (14), 339 (7), 338 (21), 203 (21), 173 (10), 161 (41), 148 (12), 136 (28) and 135 (100). (Found: C, 68.64; H, 6.39. C<sub>22</sub>H<sub>24</sub>O<sub>6</sub> requires: C, 68.75; H, 6.25%.)

β-O-Ethylcubebin [(2R, 3R, 4R)-3,4-bis-(3,4-methylenedioxybenzyl)-2β-ethoxy-furan] (5). Viscous mass,  $R_f$  0.63 (C<sub>6</sub>H<sub>6</sub>-EtOAc, 49:1, twice developed), UV  $\lambda_{max}^{MeOH}$  nm (log ε): 232 (4.01) and 286 (3.85). IR v cm<sup>-1</sup>: 2920, 1610, 1505, 1490, 1250, 1040, 930 and 8.10. CD:  $\Delta \epsilon_{240}$ . -0.45 and  $\Delta \epsilon_{288}$ - 0.61 (MeOH; c 0.08). <sup>1</sup>H NMR: see Table 1. MS m/z (rel. int): 384 (16), 339 (7), 338 (31), 203 (54), 173 (22), 162 (8), 161 (12), 145 (12), 136 (23) and 135 (100). (Found: C, 68.91; H, 6.10. C<sub>22</sub>H<sub>24</sub>O<sub>6</sub> requires: C, 68.75; H, 6.25%.)

Preparation of  $\alpha$ - and  $\beta$ -O-ethyl cubebins (4 and 5) from (-)cubebin (8). To a cooled suspension of NaH (50%, 40 mg) in dry THF (10 ml) was added (-)-cubebin (250 mg) and the mixture was stirred for 2 hr. A soln of C<sub>2</sub>H<sub>3</sub>Br in dry THF (10 ml) was then added drop-wise and stirred for 2 hr before refluxing gently for 2 hr. After usual work-up, a mixture of  $\alpha$ - and  $\beta$ -O-ethyl cubebins (205 mg) was obtained. The mixture was resolved into individual compounds by prep. TLC. The compound having lower  $R_f$  0.59 (102 mg) was identical with  $\alpha$ -O-ethyl cubebin (4) and the one with higher  $R_f$  0.63 (54 mg) with  $\beta$ -O-ethyl cubebin (5) in all respects (co-TLC, IR, NMR).

(--)-5"-Methoxyhinokinin [(2R, 3R)-2-(5"-methoxy-3",4"methylenedioxybenzyl)-3-(3',4'-methylenedioxybenzyl)butyrolactone (6). Viscous mass,  $[\alpha]_{D}^{23} - 37^{\circ}$  (CHCl<sub>3</sub>; c0.15).  $R_f$ 0.5 ( $C_6H_6$ -EtOAc, 4:1), UV  $\lambda$  (log  $\varepsilon$ ): 237 (3.85) and 285 (3.63). IR  $\nu$  cm<sup>-1</sup>: 2985, 1770, 1640, 1510, 1490, 1450, 1250, 1040 and 925. CD:  $\Delta \varepsilon_{236} - 2.6$  and  $\Delta \varepsilon_{286} - 0.43$  (MeOH; c 0.059). <sup>1</sup>H NMR:  $\delta$ 2.42-2.60 (4H, m, H-2, H-3 and H-5), 2.82-2.96 (2H, m, H-6), 3.84-3.96 (1H, m, H-4a), 3.88 (3H, s, 5"-OMc), 4.08-4.18 (1H, m, H-4b), 5.96 and 5.98 (2H each, s each,  $2 \times - \text{OCH}_2\text{O} -$ ), 6.33 (2H, s, H-2" and H-6"), 6.49 (2H, m, H-2' and H-6') and 6.72 (1H, d, J = 8 Hz, H-5'). MS m/z (rel. int): 384 (56), 249 (2), 225 (6), 222 (4), 166 (59), 165 (100), 136 (21) and 135 (53). (Found: C, 65.40; H, 5.28. C<sub>21</sub>H<sub>20</sub>O<sub>7</sub> requires: C, 65.63; H, 5.21%.)

Hemiariensin [4-acetoxy-2,3-bis-(3,4-methylenedioxybenzyl)butan-1-ol] (7). Viscous mass,  $R_f 0.3$  ( $C_6H_6$ -EtOAc, 4:1), UV  $\lambda_{meO}^{MeOH}$  nm (log  $\varepsilon$ ): 232 (4.42) and 286 (4.39). IR v cm<sup>-1</sup>: 3500, 3020, 3000, 2880, 1730, 1605, 1500, 1490, 1440, 1250, 1040, 930 and 860. CD:  $\Delta \varepsilon_{236}$  - 0.47 and  $\Delta \varepsilon_{278}$  - 0.16 (MeOH; c 0.06). <sup>1</sup>H NMR:  $\delta$ 1.58 (1H, br s, -O<u>H</u> exchangeable with D<sub>2</sub>O), 2.06 (2H, m, H-2 and H-3), 2.08 (3H, s, -OCOC<u>H<sub>3</sub></u>), 2.63 (4H, m, H-5 and H-6), 3.63 (2H, d, J = 8 Hz, H-1), 4.10 (2H, m, H-4), 5.96 (4H, s, 2 × -OC<u>H<sub>2</sub></u>O-) and 6.58-6.74 (6H, m, Ar-<u>H</u>). MS m/z (rel. int): 400 (50), 382 (1), 340 (7), 322 (3), 204 (15), 192 (9), 187 (25), 179 (6), 161 (12), 136 (33) and 135 (100). (Found: C, 66.21; H, 6.19. C<sub>22</sub>H<sub>24</sub>O<sub>7</sub> requires C, 66.0; H, 6.0%.)

Acetylation of hemiariensin (7) to ariensin (14). 7, (6 mg) was treated with  $(Ac)_2$  O and  $C_5H_5N$  and left overnight. After usual work-up, the acetylated derivative was obtained (4.5 mg) which was identical with the diacetate of dihydrocubebin, i.e. ariensin (14), in all respects (co-TLC, IR and NMR).

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