LIGNANS FROM PIPER CUBEBA

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(Revised received 28 June 1984)

Key Word Index—*Piper cubeba*; Piperaceae; lignans; (-)-cubebin; (-)-hinokinin; (-)-clusin; (-)-dihydroclusin; (-)-dihydroclusin; (-)-cubebinin; NMR.

Abstract—From the hot petrol extract of *Piper cubeba* ftuits, six lignans were isolated. Two of these, which have been obtained from a natural source for the first time, have been characterized as (2R,3R)-2-(3'',4'',5''-trimethoxybenzyl)-3-(3',4'-methylenedioxybenzyl)-1,4-butanediol [(-)-dihydroclusin] and <math>(3R,4R)-3,4-bis-(3,4,5-trimethoxybenzyl)tetra-hydro-2-furanol [(-)-cubebinin]. (-)-Cubebin, (-)-hinokinin, (-)-clusin and (-)-dihydrocubebin were also found in this plant. Only (-)-cubebin has been reported so far from this source.

INTRODUCTION

The fruits of *Piper cubeba*, commonly known as cubebs, are used in indigenous systems of medicine [1, 2]. Previous investigations report the presence of (-)cubebin (1) [3-5] as the only lignan from the fruits. However, re-investigation of the petrol extract of the fruits yielded five additional lignans, viz. (-)-hinokinin (2), (-)-clusin (3), (-)-dihydrocubebin (4), (2R,3R)-2-(3'',4'',5''-trimethoxybenzyl)-3-(3',4'-methylenedioxybenzyl)-1,4-butanediol (5) [(-)-dihydroclusin] and (3R,4R)-3,4-bis-(3,4,5-trimethoxybenzyl)tetrahydro-2-furanol (6) [(-)-cubebinin]. This is the first report of the isolation of (-)-dihydroclusin and (-)-cubebinin from a natural source. Moreover, this constitutes the first instance of a dibenzylbutyrolactol lignan in which both the aromatic rings are trisubstituted.

RESULTS AND DISCUSSION

The petrol extract on concentration deposited (-)cubebin as the major lignan, while the rest of the lignans were obtained from the filtrate in pure form by repeated column and thin-layer chromatographic separation. The known lignans, viz. (-)-cubebin [4, 5], (-)-hinokinin [6], (-)-clusin [6] and (-)-dihydrocubebin [7], were identified by comparison of their physical and spectroscopic properties with those reported together with a few interconversion reactions. Oxidation of (-)-cubebin (1) gave (-)-hinokinin (2), while its reduction yielded (-)dihydrocubebin (4). Similarly, (-)-yatein (7) was obtained by the oxidation of (-)-clusin (3) whereas the reduction of 3 gave (-)-dihydroclusin (5). The negative Cotton effects in the CD curves of all the known and the new lignans suggested the same (2R,3R) and (3R,4R)configurations in the butanediol and furanol types of lignans, respectively [8].

Compound 5, mp 97–98°, $C_{22}H_{28}O_7$, $([M]^+ m/z 404)$, $[\alpha]_{25}^{25} - 27.13°$ (CHCl₃), showed the presence of hydroxyl (3279 cm⁻¹) and methylenedioxy (922 cm⁻¹) groups in its IR spectrum. The ¹H NMR (100 MHz) spectrum suggested the presence of a 2,3-dibenzyl-1,4-butanediol system as in dihydrocubebin (4) [7]. The multiplets at $\delta 1.88$ and 2.69 were assigned to two methine and four benzylic protons, respectively. The four methylene protons of the two -CH₂OH groups appeared as a multiplet between δ 3.42 and 3.80, while the three aromatic methoxyl groups appeared as a singlet at δ 3.84. The two methylenedioxy protons were observed as a singlet at δ 5.92. The broad signals at δ 1.6 and 2.92, which were exchangeable with deuterium oxide, were attributed to two hydroxyl protons. The two-proton singlet, possibly due to meta protons, at $\delta 6.36$ indicated that one of the aromatic rings probably had a 3,4,5-trimethoxy substitution pattern. The doublets centred at $\delta 6.59 (J = 8 \text{ Hz})$ and 6.72 (J = 8 Hz) indicated the presence of orthocoupled protons in the other aromatic ring. However, this region integrated for three protons, suggesting the presence of an additional signal overlapping with one of the peaks of the doublets (appearing as an enhanced signal at δ 6.64) for the two ortho-coupled protons. Thus, this ring contained a 3,4-methylenedioxy substitution. The mass spectrum of the compound showed the $[M]^+$ peak at m/z404. The base peak at m/z 181 and the peak at m/z 135 confirmed the presence of the trimethoxybenzyl and methylenedioxybenzyl units, respectively. Other prominent peaks were observed at m/z 386 $[M - 18]^+$, 250 and 233. Moreover, this compound (5) was found to be identical (co-TLC, mp and superimposable IR) with the product obtained by lithium aluminium hydride reduction of clusin (3), of known (3R,4R) absolute configuration. Therefore, compound 5 was identified as (2R, 3R)-2-(3",4",5"-trimethoxybenzyl)-3-(3',4'-methylenedioxybenzyl)-1,4-butanediol. It has been named (-)-dihydroclusin. This compound has also been prepared by lithium aluminium hydride reduction of (-)-yatein (7) [9].

Compound 6, viscous mass, $C_{24}H_{32}O_8$, $([M]^+ m/z 448)$, $[\alpha]_{25}^{25} - 23.33^{\circ}$ (CHCl₃), indicated the presence of a hydroxyl group (3450 cm⁻¹) in its IR spectrum. Since the 60 MHz ¹H NMR spectrum of 6 was unsatisfactory, the same was repeated on a 500 MHz instrument. This spectrum, in general, indicated that the compound belonged to the dibenzylbutyrolactol class of lignans. The singlets at $\delta 3.80$, 3.82 and 3.84 (all integrating for 18 protons) were assigned to six aromatic methoxyl groups. The four benzylic and two methine protons gave rise to a



- $\begin{array}{ccc} 1 & R^{1} + R^{2} = R^{4} + R^{5} = -OCH_{2}O ; \\ R^{3} = R^{6} = H \end{array}$
- **3** $R^1 + R^2 = -OCH_2O -; R^3 = H;$ $R^4 = R^5 = R^6 = OMe$
- **6** $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = OMe$



- 2 $R^{1} + R^{2} = R^{4} + R^{5} = -OCH_{2}O -;$ $R^{3} = R^{6} = H$ 7 $R^{1} + R^{2} = -OCH_{2}O -;$ $R^{3} = H$
- $R^4 = R^5 = R^6 = OMe$
- **8** $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = OMe$



5 $R^1 = R^2 = R^3 = OMe$

complex multiplet between $\delta 2.04$ and 2.85. However, the hemiacetal proton gave rise to a set of signals, viz. a singlet at $\delta 5.27$ and a doublet at $\delta 5.29$ (J = 5 Hz), integrating in total for one proton. This suggested that in all probability the compound existed as a mixture of epimers. This observation was further confirmed by the appearance of triplets at $\delta 3.62 (J = 8 \text{ Hz}), 4.03 (J = 8 \text{ Hz}) \text{ and } 4.14 (J$ = 8 Hz) due to two methylene protons of the furanol ring. Similarly, the hydroxyl proton gave rise to two broad signals at δ 1.93 and 3.05. The aromatic protons produced five singlets which integrated for four protons. The appearance of signals at two different places for the same protons in the NMR spectrum of the mixture of epimers has been reported in the case of other dibenzylbutyrolactol lignans, such as 6',6"-dinitrocubebin [10] and carissanol [11]. The latter compound has been shown to exist as a mixture of epimers on the basis of ¹H NMR and ¹³C NMR spectral data.

In view of the above observations, the ¹³C NMR (broad-band decoupling) spectrum of the compound was studied. The assignments of the chemical shifts were made by comparison with the data reported for similar types of compounds [6, 9]. As expected, the carbon atoms of the dibenzylbutyrolactol skeleton gave rise to two sets of closely spaced signals. The signals at δ 153.31 (C-3', C-3", C-5' and C-5" carbons), 106.77 and 107.06 (C-2', C-2", C-6' and C-6" carbons) strongly indicated a 3,4,5-trimethoxy aromatic substitution in this compound. This was further confirmed by the appearance of signals at δ 56.51 (C-3', C-3", C-5' and C-5" methoxyl carbons) and 60.66 (C-4' and C-4" methoxyl carbons). This was based on the fact that in compounds having three methoxyl groups in consecutive disposition, the signal for the central methoxyl group is shifted downfield by $ca \, \delta 4.6$ as compared with those for the neighbouring ones [12]. Thus, in yatein (7) [9], the C-4" methoxyl group appears at $\delta 60.8$, while the C-3" and C-5" ones are seen at δ 56.1.

In order to establish the positions of the methoxyl groups in the aromatic rings unambiguously, the compound was oxidized using silver carbonate in toluene. The resultant product (8) showed in its IR spectrum absorption at 1770 cm^{-1} which confirmed the presence of a γ -lactonic carbonyl group. The ¹H NMR (500 MHz) spectrum of the compound showed a complex multiplet

between $\delta 2.45$ and 2.65 which could be assigned to the two methine and C-5 benzylic protons. The C-6 benzylic protons gave rise to a doublet at $\delta 2.90$ (J = 6.7 Hz). The two C-4 methylene protons produced triplets at $\delta 3.83$ (J = 6.7 Hz) and 4.13 (J = 6.7 Hz). Three singlets were observed at $\delta 3.75$, 3.76 and 3.77 integrating for 18 protons of the six aromatic methoxyl groups. The aromatic protons gave rise to two singlets at $\delta 6.16$ and 6.33 of equal intensities, each integrating for two protons. These protons were found to be *meta*-oriented. This was established by studying the ¹H NMR spectrum of compound **8** in deuterated benzene.

It is well known that on changing the solvent from chloroform to benzene, the methoxyl groups attached to an aromatic ring experience an upfield shift, $\Delta = \delta_{\text{CDCl}_3} - \delta_{\text{C}_6\text{D}_6} [13]. \text{ This shift is quite large } (> \delta 0.3)$ when the methoxyl group has an ortho-hydrogen and is significantly small when it is flanked on both sides by a methoxyl or other groups. In the present study, when C_6D_6 was used as solvent, the methoxyl resonances in compound 8 were observed at δ 3.43 (6 H), 3.46 (6 H), 3.78 (3 H) and 3.82 (3 H). By comparing these values with those observed in CDCl₃, it was seen that four methoxyl groups underwent a distinct upfield shift, as was evident from the singlets at δ 3.43 and 3.46. These resonances were attributed to the C-3', C-3", C-5' and C-5" methoxyl groups. On the other hand, the singlets at $\delta 3.78$ and 3.82 were assigned to the C-4' and C-4" methoxyl groups since these would be expected to experience only negligible changes in their chemical shift values. The mass spectrum of compound 8 showed a $[M]^+$ at m/z 446, while the base peak at m/z 181 confirmed the presence of trimethoxybenzyl units in the molecule. Other prominent ions were observed at m/z 265, 238, 223, 219 and 182.

The absolute configuration of compound 6 was established as (3R,4R), since it displayed negative Cotton effects at 244 and 270 nm, as in the case of cubebin (1) [8] and yatein (7) [9]. Hence, 8 possessed a (2R,3R) configuration and was characterized as (2R,3R)-2,3-bis-(3,4,5trimethoxybenzyl)butyrolactone. Compound 6 could thus be designated as (3R,4R)-3,4-bis-(3,4,5-trimethoxybenzyl)tetrahydro-2-furanol. These two new compound's have been named cubebininolide and cubebinin, respectively.

EXPERIMENTAL

Mps are uncorr. ¹H NMR (60, 100 and 500 MHz) and ¹³C NMR (125.775 MHz): CDCl₃. MS (70 eV) direct insertion. $[\alpha]_D^{25}$: CHCl₃. UV: MeOH. CD: MeOH. IR: KBr, CHCl₃ or as film. Chromatographic separations were carried out on neutral Al₂O₃. TLC was performed on silica gel G using C₆H₆-EtOAc and CHCl₃-Me₂CO as solvent systems. Spots were detected on TLC in UV (254 nm), exposing the plates in I₂ vapour and heating to 100° in an oven after spraying with 10% H₂SO₄.

Extraction and separation of compounds. The dried fruits of P. cubeba (2 kg) were obtained from M/s. United Chemicals and Allied Products, Calcutta. The fruits (1.6 kg) were ground and then extracted with petrol in a Soxhlet for 56 hr. Removal of the solvent under red. pres. furnished a resinous mass (28 g). This was then triturated with EtOH whereby a white amorphous solid (4 g) separated out. This crystallized from dry EtOH to give (-)cubebin (1) (3.5 g). The EtOH soluble portion was coned in vacuo to a resinous mass (24 g). This on repeated CC over neutral Al₂O₃, using increasing polarity of solvents from petrol to EtOAc followed by prep. TLC on silica gel, yielded compounds 1-6 in pure form. These were characterized as (-)-cubebin (1) [4, 5], (-)-hinokinin (2), (-)-clusin (3) [6], (-)-dihydrocubebin (4) [7], (-)-dihydroclusin (5) and (-)-cubebinin (6).

(-)-Dihydroclusin [(2R,3R)-2-(3",4",5"-trimethoxybenzyl)-3-(3',4'-methylenedioxybenzyl)-1,4-butanediol] (5). Mp 97-98°, [α]²⁵_D - 27.13° (CHCl₃; c 0.24). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ε): 229 (4.09) and 285 (3.51). IR $\nu_{\text{max}}^{\text{MeT}}$ cm⁻¹: 3279, 2941, 1587, 1235, 1031, 922, 806 and 772. CD $\Delta \varepsilon_{234}$ - 1.34 and $\Delta \varepsilon_{276}$ - 0.19 (MeOH; c 0.05). ¹H NMR (100 MHz): δ 1.88 (2H, m, -CH₂-CH-CH-CH₂-), 2.69 (4H, m, 2 Ar-CH₂-), 3.42-3.80 (4H, m, 2-CH₂-OH), 3.84 (9H, s, Ar-OCH₃), 5.92 (2H, s, -O-CH₂-O), 6.36 (2H, s, C-2" and C-6" protons), 6.59 (2H, d, J = 8 Hz, C-5' or C-6' proton), 6.72 (2H, d, J = 8 Hz, C-5' or C-6' proton), 6.64 (1H, s, C-2' proton), 1.6 (1H, br, -OH), 2.92 (1H, br, -OH). MS m/z (rel. int.): 404 [M]⁺ (41), 386 (10), 250 (2.7), 233 (2.7), 225 (3.6), 182 (100), 181 (98), 136 (16) and 135 (56). (Found: C, 65.18; H, 6.85. C₂₂H₂₈O₇ requires: C, 65.35; H, 6.93 %.)

LiAlH₄ reduction of (-)-clusin (3) to (-)-dihydroclusin (5). A soln of 3 (30 mg) in dry Et₂O (10 ml) was added dropwise to a suspension of LiAlH₄ (30 mg) in dry Et₂O (10 ml) under dry conditions with stirring. The mixture was stirred further for 4 hr at room temp. and then poured into H₂O at 0°. After acidifying the mixture carefully with HCl (7%), it was extracted with Et₂O (3 × 40 ml). The combined organic phase was washed with H₂O, dried (Na₂SO₄) and then concd. The product (22 mg) thus obtained was purified by prep. TLC (silica gel, CHCl₃-Me₂CO, 4:1, R_f 0.32) followed by crystallization (C₆H₆-petrol) to yield pure compound 5 (10 mg). This product was homogeneous with (-)-dihydroclusin in all respects (co-TLC, mp, mmp, IR).

(-)-Cubebinin [(3R,4R)-3,4-bis-(3,4,5-trimethoxybenzyl)tetrahydro-2-furanol] (6). Viscous mass, $[\alpha]_{D}^{25} - 23.33^{\circ}$ (CHCl₃; c 0.3). UV λ_{max}^{MeOH} nm (log ϵ): 226 (4.24) and 270 (3.20). IR $\nu_{max}^{CHCl_3}$ cm⁻¹: 3450, 1613, 1515, 1460, 1250 and 1010. CD: $\Delta \epsilon_{244} - 1.005$ and $\Delta \epsilon_{270} - 0.173$ (MeOH; c 0.05). ¹H NMR (500 MHz): $\delta 2.04-2.85$ (6H, m, 2 Ar-CH₂-, -CH₂-CH-CH-CH₂-), 3.62, 4.03 and 4.14 (2H, t each, J = 8 Hz, C-5 methylene protons), 3.80 (3H, s, Ar-OCH₃), 3.82 (9H, s, Ar-OCH₃), 3.84 (6H, s, Ar-OCH₃), 5.27 and 5.29 (1H, s and d, J = 5 Hz, respectively, C-2 proton), 6.28, 6.35, 6.36, 6.40 and 6.47 (4H, s each, Ar-H), 1.93 and 3.05 (1H, br each, -OH). ¹³C NMR (125.775 MHz): δ 38.96, 39.70 and 40.0 (C- 6, C-7), 43.12 and 46.10 (C-4), 52.05 and 53.53 (C-3), 56.51 (C-3', C-3", C-5", C-5" OMe carbons), 60.66 (C-4', C-4" OMe carbons), 72.26 and 72.56 (C-5), 99.03 and 103.64 (C-2), 106.77 and 107.06 (C-2', C-2", C-6', C-6"), 135.02, 135.47 and 135.49 (C-1', C-1"), 135.91, 136.20, 137.54 and 137.69 (C-4', C-4"), 153.31 (C-3', C-3", C-5', C-5"). MS m/z (rel. int.): 448 [M]⁺ (41.5), 430 (24.5), 249 (32), 182 (13.6) and 181 (100). (Found: C, 64.51; H, 7.09. $C_{24}H_{32}O_8$ requires: C, 64.29; H, 7.14%)

Oxidation of (-)-cubebinin (6) to (-)-cubebininolide [(2R,3R)-2,3-bis-(3,4,5-trimethoxybenzyl)butyrolactone (8) [14]. To a soln of 6 (20 mg) in toluene (10 ml), Ag₂CO₃ (100 mg) was added and the mixture heated slowly under N_2 to its bp (110°) with stirring. As the temp. neared the bp, the colour of the soln changed from olive-green to grey and finally to black within 5 min. The cooled reaction mixture was filtered and the solvent removed in vacuo to obtain a viscous mass (18 mg). This was purified by prep. TLC (silica gel, CHCl₃-Me₂CO, 9:1, R_f 0.53) to yield pure (-)cubebininolide (8), (12 mg) as a viscous mass, $\left[\alpha\right]_{D}^{25} - 17.6^{\circ}$ (CHCl₃; c 0.23). UV 1 MeOH nm (log s): 226 (4.23) and 272 (3.3). IR vfim cm⁻¹: 1770, 1587, 1504, 1460, 1333 and 1130. ¹H NMR (500 MHz): $\delta 2.45-2.65$ (4H, m, C-5 benzylic protons, $-CH_2-CH_2-CH_2-CH_2-$, 2.90 (2H, d, J = 6.7 Hz, C-6 benzylic protons), 3.75 (6H, s, Ar-OCH₃), 3.76 (6H, s, Ar-OCH₃), 3.77 (6H, s, Ar-OCH₃), 3.83 and 4.13 (2H, t each, J = 6.7 Hz, C-4 methylene protons), 6.16 (2H, s, Ar-H), 6.33 (2H, s, Ar-H). MS m/z (rel. int.): 446 [M]⁺ (64), 265 (4.5), 238 (1.5), 223 (3.6), 219 (3), 182 (60) and 181 (100). (Found C, 64.81; H, 6.43. C24H30O8 requires: C, 64.57; H, 6.73 %.)

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