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POLYPHENOLS FROM *STEMONOPORUS* SPECIES

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Key Word Index—*Stemonoporus affinis*; *S. cordifolius*; *S. elegans*; *S. kanneliensis*; *S. lancifolius*; *S. oblongifolius*; Dipterocarpaceae; copalliferol *A*; stemonoporol; vaticaffinol; antimicrobial activity.

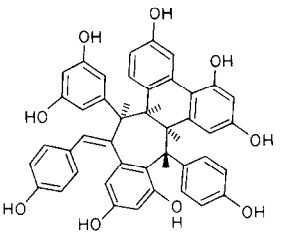
Abstract—Methanol extracts of the bark of six *Stemonoporus* species have been investigated. A new polyphenol, stemonoporol, has been isolated from four species. The polyphenols, copalliferol A and vaticaffinol are reported for the second time. Formic acid treatment converted stemonoporol to copalliferol A.

INTRODUCTION

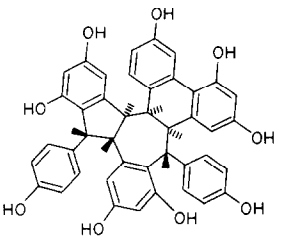
The genus *Stemonoporus* is endemic to Sri Lanka and 15 species belonging to this genus are recorded [1]. In an earlier publication [1], we reported the isolation of several triterpenoids from benzene extracts of the bark and timber of six *Stemonoporus* species. The chemical similarities of the phytoconstituents were very striking. The chemical investigation of the methanol extracts of the bark of these species is reported in this paper.

RESULTS AND DISCUSSION

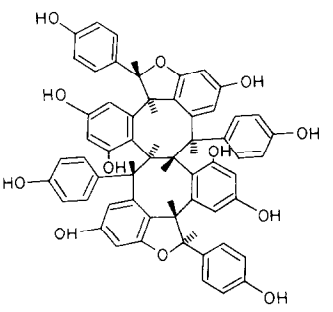
Column chromatographic separation followed by prep. TLC purification of the methanol extracts of *S. affinis*, *S. elegans*, *S. kanneliensis* and *S. oblongifolius* gave, in each case, the same polyphenol, stemonoporol (**1**). Stemonoporol was assigned a molecular formula of $C_{42}H_{32}O_9$ (M^+ 680.1979). Its IR spectrum had no absorption bands in the region 1740–1640 cm^{-1} but contained strong absorption bands at 3250 cm^{-1} due to hydroxyl group(s). The ^1H NMR spectra of the dimethyl sulphate methylation product and of the acetylation product of stemonoporol indicated the presence of nine hydroxyl substituents. The ^1H NMR spectrum of stemonoporol had complex aromatic proton signals at δ 5.6–7.7 integrating for 18 aromatic protons, complex benzylic proton signals at δ 3.2–4.9 (4H) and an olefinic proton signal at δ 5.2. Treatment of stemonoporol with formic acid gave another isomeric polyphenol. The latter did not have the olefinic proton signal at δ 5.2 in its ^1H NMR spectrum. However, the benzylic protons at δ 3.5–4.9 integrated for 6H and the complex aromatic signals at δ 5.6–6.6 integrated for 17 protons. The latter polyphenol was found to be identical with copalliferol A (**2**) isolated in this study. Stemonoporol (**1**) unlike copalliferol A (**2**) was UV fluorescent. These data show that both these polyphenols are related to one another and that stemonoporol (**1**) is cyclized in the presence of acids to copalliferol A (**2**). The cyclization involves addition of H^+ to the olefinic bond of stemonoporol (**1**) followed by electrophilic substitution at the activated aromatic site.



1 Stemonoporol



2 Copalliferol A



3 Vaticaffinol

Copalliferol A was eluted from the column immediately after stemonoporol. High resolution mass spectrometry of this polyphenol gave the molecular formula as $C_{42}H_{32}O_9$ (M^+ 680.2075), i.e. isomeric with stemonoporol. Methylation of the polyphenol with dimethyl sulphate gave a nonamethyl ether indicating the presence of nine hydroxyl groups in the polyphenol. The ^1H NMR spectrum of the polyphenol showed the presence of 17 aromatic protons (δ 5.6–6.6) and six benzylic protons (δ 3.5–4.9). The polyphenol did not have any IR absorption bands in the region 1640–1740 cm^{-1} showing the absence of any carbonyl groups. Comparison of this polyphenol with copalli-

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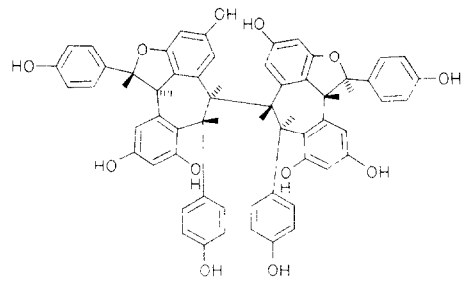
ferol A isolated by Sultanbawa *et al.* [2] from *Vateria copallifera* (Dipterocarpaceae) showed that both polyphenols were similar (IR, ^{1}H NMR, co-TLC). Copalliferol A was found in all the *Stemonoporus* species which contained stemonoporol.

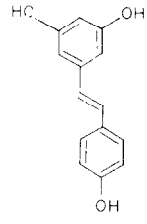
Two *Stemonoporus* species viz. *S. cordifolius* and *S. lancifolius* did not have the above two polyphenols. However, column chromatographic separation followed by prep. TLC purification of their methanol extracts yielded yet another polyphenol which has been identified as vaticaffinol (**3**) from the following data. Complete methylation with dimethyl sulphate gave a decamethylated product, $C_{66}H_{62}O_{12}$ (M^+ 1046.4241). The parent polyphenol was assigned the formula $C_{56}H_{42}O_{12}$. Two oxygen atoms in the polyphenol should be present either as two hindered hydroxyl groups or as ether functions since the IR spectrum showed no absorption bands in the region 1640–1740 cm^{-1}. Absence of any hydroxyl absorption bands in the IR spectrum of the decamethyl ether confirmed the presence of only ten hydroxyl groups in the natural product. The ^{1}H NMR spectrum of the polyphenol showed the presence of 24 aromatic protons (δ 6.0–7.5) and eight benzylic protons (δ 3.8–5.8). Comparison with the other polyphenols isolated indicated that it was identical (co-TLC, IR, ^{1}H NMR) with vaticaffinol isolated from *V. affinis* by Sultanbawa *et al.* [3].

The percentage yields of polyphenols isolated from the six *Stemonoporus* species are given in Table 1. Hopeaphenol was the first polyphenol of this nature to be isolated and it was assigned [4] the symmetrical structure **4** using evidence from the mass spectrum which had $[M/2]^+$ as the base peak. Structure **4** indicates hopeaphenol to be a resveratrol (**5**) tetramer. Vaticaffinol (**3**) is isomeric with hopeaphenol (**4**) and Sultanbawa *et al.* [3] used the resveratrol oligomerization hypothesis to postulate the structure for vaticaffinol. Stemonoporol (**1**) and copalliferol A are both resveratrol trimers. A resveratrol dimer, ϵ-viniferin (**6**), has been shown to be a phytoalexin and was isolated from infected grapevine (*Vitis vinifera*) [5]. However, the yields of the polyphenols isolated in this study (Table 1) indicate that these are natural products and not phytoalexins like ϵ-viniferin.

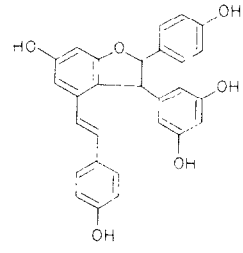
The methanol extracts of all the *Stemonoporus* species showed antibacterial activity against three strains of micro-organism: Oxford staphylococcus, *Escherichia coli* and yeast. Of the three different polyphenols isolated, only copalliferol A showed any significant antimicrobial activity.



4 Hopeaphenol



5 Resveratrol



6 ϵ-Viniferin

EXPERIMENTAL

The barks of the *Stemonoporus* species were collected from the Kanneliya rain-forest, south of Sri Lanka. They were milled and extracted with petrol and then with MeOH. The results of the investigation on the petrol extracts were published earlier [1].

The MeOH extracts of *S. affinis*, *S. elegans*, *S. kanneliensis* and *S. oblongifolius* when processed as follows gave stemonoporol.

Isolation of stemonoporol. The MeOH extract (20 g) was chromatographed over Si gel (300 g). Elution with C_6H_6–Me_2CO (1:1) afforded crude stemonoporol (150 mg). This was fluorescent under UV and was purified by prep. TLC (Si

Table 1. Distribution of polyphenols in *Stemonoporus* species

Name of plant	Amount†			
	Bergenin	Stemonoporol	Copalliferol A	Vaticaffinol
S. affinis	2.6 [1]	1.8	2.8	—
S. cordifolius	—	—	—	5.1
S. elegans	—	1.4	2.0	—
S. kanneliensis	1.5	1.9	2.9	—
*S. lancifolius**	1.8 [1]	—	—	5.3
S. oblongifolius	—	3.3	1.8	—

*Originally identified (1976) as a *Stemonoporus* species it is now considered to be a *Hopea-Balanocarpus* species within the family Dipterocarpaceae.

†Percentage dry wt with respect to the amount of plant material used.

gel G, C_6H_6–Me_2CO, 1:1, to give the pure polyphenol (93 mg), mp > 300°, $[\alpha]_D = -5.58°$ (MeOH). Found $[M]^+$ 680.1979, $C_{42}H_{32}O_9$ requires $[M]^+$ 680.2046; IR ν_{max}^{KBr} cm^{-1}: 3000–3600, 1600, 1430–1500, 1330, 1230, 1150, 1000, 830; UV λ_{max}^{MeOH} nm: 283 (log ϵ 3.4065); $\lambda_{max}^{MeOH-NaOH}$ 296 (3.6284); ^1H NMR (60 MHz, CD_3COCD_3): δ 8.00 (9H, *br s*, OH), 5.6–7.7 (18H, aromatic), 5.2 (1H, *br s*, olefinic), 3.2–4.9 (4H, benzylic); MS *m/z* (rel, int.): 680(95), 678(100), 662(40), 586(60), 584(65), 575(60), 573(68), 571(62), 482(45), 481(45), 461(42), 347(38), 331(38), 199(40).

Nonamethyl stemonoporol. A soln of stemonoporol (93 mg) in Me_2CO (5 ml) was heated under reflux for 8 hr under dry conditions with Me_2SO_4 (0.1 ml) and K_2CO_3 (20 mg). The product was purified by prep. TLC to yield the pure methyl derivative (62 mg), mp 138–140°, ^1H NMR (60 MHz, $CDCl_3$): δ 5.8–7.8 (18H, aromatic), 5.2 (1H, olefinic), 3.2–4.8 (31H, 9 × OMe + 4 benzylic protons).

Reaction of stemonoporol with HCO_2H. Stemonoporol (60 mg) was dissolved in HCO_2H (1 ml) and the mixture was heated in an oil bath for 6 hr. The product after the usual work-up was purified by prep. TLC to give a product which was identified as copalliferol A (co-TLC, IR, ^1H NMR and specific rotation).

Isolation of copalliferol A. After separation of stemonoporol, the column on elution with C_6H_6–Me_2CO (1:1) gave impure copalliferol A. Prep. TLC gave pure copalliferol A, mp > 300°, $[\alpha]_D + 115°$ (MeOH). Found $[M]^+$ 680.2075, $C_{42}H_{32}O_9$ requires $[M]^+$ 680.2046. UV λ_{max}^{EtOH} nm: 282 (log ϵ 2.9380), $\lambda_{max}^{EtOH-NaOH}$ 288 (3.0229); IR ν_{max}^{KBr} cm^{-1}: 3000–3600, 1600, 1500, 1440, 1330, 1210, 1100, 1010, 830 and 670; ^1H NMR (60 MHz, CD_3COCD_3): δ 7.8–8.0 (OH), 5.6–6.6 (17H, aromatic), 3.5–4.9 (6H, benzylic); MS *m/z* (rel. int.): 680(10), 482(40), 350(70), 348(72), 332(69), 226(40), 216(51).

Nonamethyl copalliferol A. A soln of copalliferol A (122 mg) in Me_2CO (10 ml) was heated under reflux with Me_2SO_4 (0.1 ml) and K_2CO_3 (20 mg). The product was isolated in the usual manner and was purified by prep. TLC to give the pure methyl derivative, mp 146–147°, $[\alpha]_D + 92.8°$ ($CHCl_3$); ^1H NMR (60 MHz, $CDCl_3$): δ 5.6–7.4 (17H, aromatic), 4.0–4.9 (6H, benzylic), 3.4–3.8 (27H, 9 × OMe).

Nona-acetate of copalliferol A. A soln of copalliferol A (102 mg) in C_5H_5N (5 ml) was heated in a boiling water bath overnight. The product was purified by prep. TLC and the pure nona-acetate, mp 174–176° was isolated. ^1H NMR (60 MHz, $CDCl_3$): δ 5.8–7.2 (17H, aromatic), 3.6–4.8 (6H, benzylic), 1.8–2.6 (27H, 9 × –OCOMe).

Isolation of vaticaffinol. The MeOH extracts of *S. cordifolius* and *S. lancifolius* were separated on a column of Si gel. Elution with C_6H_6–Me_2CO (1:1) gave crude vaticaffinol. Prep. TLC gave vaticaffinol, mp > 300°, $[\alpha]_D$ −22.5° (MeOH). Found $[M]^+$ 906, $C_{56}H_{42}O_{12}$ requires 906.3246; IR ν_{max}^{KBr} cm^{-1}: 2200–2700, 1600, 1440, 1330, 1230, 1160, 1000, 830; UV λ_{max}^{EtOH} nm: 282 (log ϵ 4.44); ^1H NMR (60 MHz, CD_3COCD_3): δ 8.2 (1OH, –OH), 6.0–7.5 (24H, aromatic), 3.8–5.8 (8H, benzylic).

Decamethylvaticaffinol. A soln of vaticaffinol (92 mg) in Me_2CO (5 ml) was heated with Me_2SO_4 (0.1 ml) and K_2CO_3 (20 mg). The reaction product was separated by prep. TLC and the pure methylated vaticaffinol (72 mg) was isolated, mp 160–162°, $[\alpha]_D + 20.8°$ ($CHCl_3$). Found $[M]^+$ 1046.4252; $C_{66}H_{62}O_{12}$ requires 1046.4241; ^1H NMR (60 MHz, $CDCl_3$): δ 6.0–7.4 (24H, aromatic), 3.2–5.8 (38H, benzylic and OMe).

Vaticaffinol deca-acetate. A soln of vaticaffinol (102 mg) in C_5H_5N (5 ml) and Ac_2O (0.1 ml) were left at room temp. overnight. The product was isolated in the usual manner to give the pure acetate, mp 155–157°, $[\alpha]_D$ −33.9° ($CHCl_3$); ^1H NMR (60 MHz, $CDCl_3$): δ 6.0–7.6 (24H, aromatic), 3.7–5.8 (benzylic), 2.30 (30H, –OAc).

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