# Polyphenols from Dipterocarp Species. Copalliferol A and Stemonoporol

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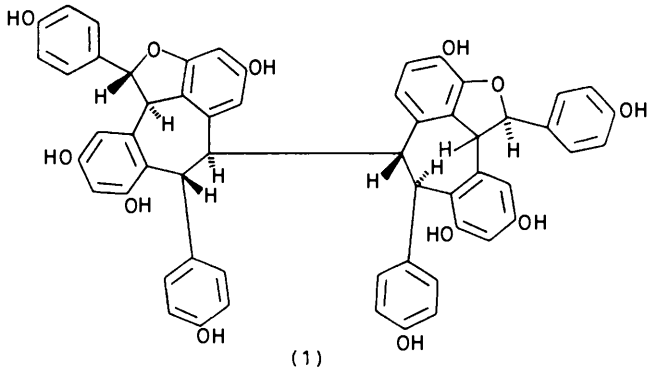
A new antibacterial polyphenol, copalliferol A, has been isolated from four dipterocarp species, *Hopea cordifolia*, *Hopea brevipetiolaris* (=*Balanocarpus zeylanicus*), *Shorea stipularis* and *Vateria copallifera*. The latter two species also contained another polyphenol, stemonoporol, which when treated with formic or toluene-*para*-sulphonic acid is transformed into copalliferol A.

Hopeaphenol ($C_{56}H_{42}O_{12}$) (1) was the first polyphenol isolated [1] from a dipterocarp species. It was regarded as a tetramer of 3,5,4'-trihydroxystilbene (resveratrol) ($C_{14}H_{12}O_3$) and its structure (1) was established by *X*-ray methods. The presence of hopeaphenol in other dipterocarp species was confirmed by Seshadri *et al.*[2] The dimer and trimer of *trans*-resveratrol have been isolated [3] from infected grape vine leaves and they have been shown to be phytoalexins. In this study the chemical investigation of the cold acetone bark extracts of the following Dipterocarp species were undertaken: *Vateria copallifera* (Retz.)Alston, *Balanocarpus zeylanicus* (= *Hopea brevipetiolaris* (Thw.) Ashton), *Shorea stipularis* Thw., and *Hopea cordifolia* (Thw.) Trimen. All the extracts contained the new polyphenol [4] copalliferol A (2). The bark extractives of *Vateria copallifera* and *Shorea stipularis* also contained another new polyphenol called stemonoporol (3) which is isomeric with copalliferol A (2). Stemonoporol (3) was isolated independently by Samaraweera *et al.*[5] in this laboratory from several *Stemonoporus* species of Sri Lanka. Stemonoporol (3) is an unstable polyphenol which undergoes conversion into copalliferol A (2) when treated with formic acid or toluene-*p*-sulphonic acid. This paper deals with the structure of copalliferol A (2).



(1)

## Results and Discussion

Copalliferol A (2) and stemonoporol (3) had very close t.l.c. $R_F$ values and when present together were separated by p.t.l.c. The polar polyphenol was copalliferol A (2), m.p. >300 °C (decomp.), $[\alpha]_D^{25}$ +115.6° (MeOH) and $M^+$ 680.2075 ($C_{42}H_{32}O_9$). It did not give any of the characteristic colour reactions of flavanoids, triterpenoids or sugars. The formation of a nonamethyl ether {m.p. 145—147 °C, $[\alpha]_D^{25}$ +92.8° ($CHCl_3$), $M^+$ 806.3600 ($C_{51}H_{50}O_9$)} and a nona-acetate, m.p. 176—178 °C showed that all the oxygen atoms were present as hydroxy-groups. The u.v. spectrum ($\lambda_{max.}$ (EtOH) 282 nm (($\varepsilon$ 867) was indicative of an unconjugated phenolic chromophore(s) and the spectrum was unaltered by the addition of sodium acetate-boric acid showing the absence of any *o*-dihydroxy-groups. The i.r. spectrum contained no carbonyl absorption but showed a broad hydroxy-band at 3 250 $cm^{-1}$, aromatic absorption at 1 600 $cm^{-1}$, and a prominent band at 830 $cm^{-1}$ suggestive of the presence of 1,4-disubstituted benzene system(s). Copalliferol A (2) did not give any recognisable products on degradation (conc. $HNO_3$ and alkali fusion). The molecular formula ($C_{42}H_{32}O_9$) indicated that copalliferol A (2) like hopeaphenol could be an oligomer of resveratrol ($C_{14}H_{12}O_3$) units. Copalliferol A (2) could be formed from three resveratrol units. The $^{13}C$ n.m.r. spectrum of copalliferol A (2) showed: 6 aliphatic doublets ($\delta_c$ 43—64 p.p.m.), 9 phenolic carbon atoms (153—160 p.p.m.), 10 aromatic doublets (assigned to 17 atoms), and 10 quaternary carbon singlets.

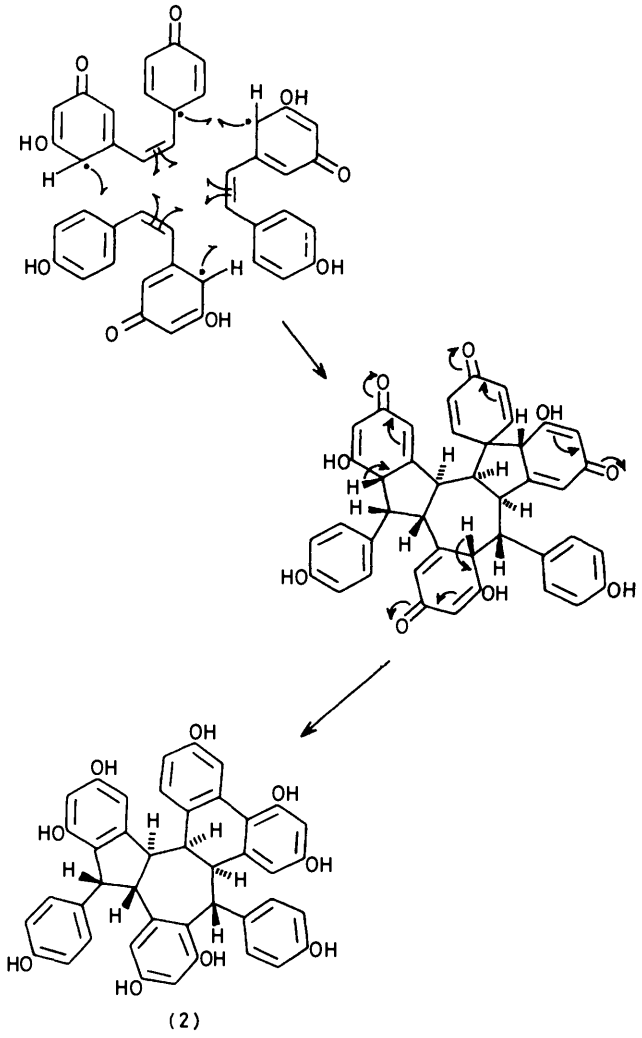
The $^1H$ n.m.r. spectra at high frequency (270, 360 MHz) showed resonance due to the presence of 9 hydroxy-groups, 17 aromatic protons, and 6 aliphatic methine protons in the region $\delta_H$ 3.5—4.9. The methine protons at $\delta_H$ 4.56 (1 H) and 4.63 (1 H), appeared as doublets coupled to one another (*J* 3.75 Hz). Three other methine protons at $\delta_H$ 3.51, 3.86, and 4.90 appeared as doublet (*J* 6.75 Hz), double doublet (*J* 6.75 Hz and *J* 2.0 Hz) and doublet (*J* 2.0 Hz) respectively. One of the methine protons appeared as a singlet at $\delta_H$ 4.10. The coupling constants together with the chemical shifts suggested that the methine protons were from different rings.

The presence of these 6 methine protons show that they could have arisen from 6 olefinic protons of the three resveratrol units during oligomerisation (Scheme 1). The presence of only 17 aromatic protons out of a total of 21 aromatic protons from the three resveratrol units shows that phenolic oxidative coupling would have taken place at 4 aromatic carbons as shown in Scheme 1.

Eighteen possible structures for copalliferol A can be written at this stage, eight with 5-7-6 membered rings of copalliferol A linearly fused and ten with 5-7-6-membered rings angularly fused. The presence of the 5-7-6 membered ring explains the six methine proton signals in the $^1H$ n.m.r. spectrum. Angular fusion of the 5-7-6 membered rings is preferred because this explains the mass spectral fragmentations of copalliferol A (Scheme 2).

Out of the 10 angularly fused structures only (2) is compatible with the following: (*a*) coupling of the six methine protons; (*b*) the $^{13}C$ n.m.r. signals; (*c*) the possible formation of copalliferol A from the oxidative coupling of three resveratrol units.

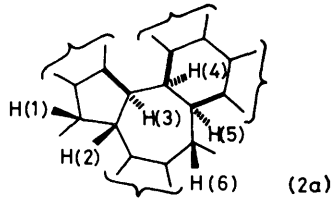
The stereochemistry of the methine protons as shown in (2) was obtained from the coupling constants. It was found from



(2)

**Scheme 1.** Oligomerisation of resvertrol units to produce copalliferol A (2)

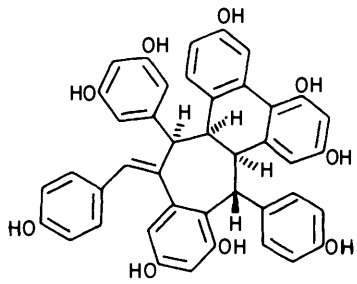
a Dreiding model that the *cis-trans-cisoid-cis-trans* stereochemistry of the 5-7-6 membered ring (2a) would best explain the coupling constants of the methine protons: $\delta_H$ 3.51(d,1-H), 3.86(dd,2-H), 4.90(d,3-H), 4.10(s,4-H), 4.63(d, 5- or 6-H) and 4.55(d,6-H or 5-H).



(2a)

3·51(d, 1-H), 3·86(dd,2-H), 4·90(d, 3-H), 4·10(s,4-H), 4·63(d, 5-or 6-H) and 4·55(d,6-H or 5-H)

Decoupling experiments showed that 1-H, 2-H, and 3-H are adjacent to each other and so are 5-H and 6-H; 4-H appeared as a singlet since the distorted conformation made it impossible to couple with 3-H or 5-H. The $^{13}C$ n.m.r. assignments are given in Scheme 3.



(3)

Stemonoporol (3) was less polar than copalliferol A (2). It too decomposes at 300 °C, $[\alpha]_D$ −5.6 (MeOH) and gave $M^+$ 680.1979 ($C_{42}H_{32}O_9$) and hence is isomeric with copalliferol A (2). Stemonoporol (3) also formed a nonamethyl ether, m.p. 138—40 °C and a nona-acetate, m.p. 166—168 °C. The u.v. spectrum showed $\lambda_{max.}$ (MeOH) 283 nm (ε 2 550) and was very similar to that of copalliferol A. The presence of phenolic OH group(s) was indicated by the bathochromic shift observed in the u.v. spectrum when NaOH was added. Both copalliferol A (2) and stemonoporol (3) did not answer the neutral iron(III) chloride test for phenols. I.r. spectra of both polyphenols were remarkably similar.

Treatment of stemonoporol (3) with either formic acid or toluene-*p*-sulphonic acid gave copalliferol A (2). Stemonoporol (3) isolated was identical (t.l.c., i.r., $^1H$ n.m.r.) to the polyphenol isolated from *Stemonoporus* species by Samaraweera *et al.*[5] As shown by these authors, protonation of the olefinic bond by the acid followed by an electrophilic attack on the reactive aromatic nucleus could convert stemonoporol (3) to copalliferol A (2). The percentages of the polyphenols isolated from the different dipterocarp species are given in the Table.

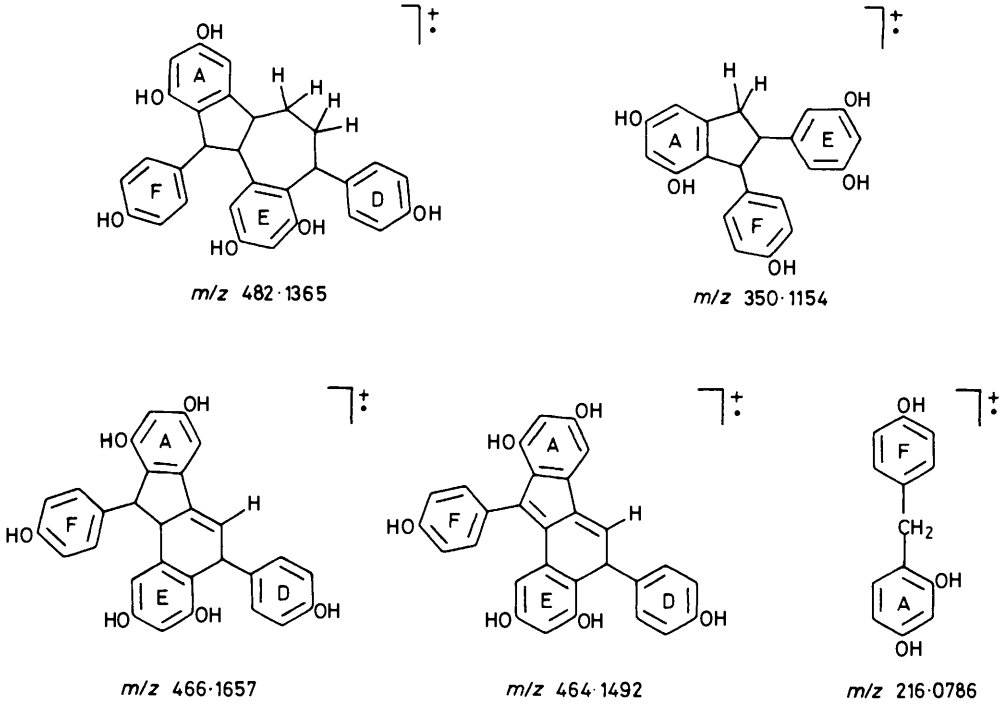
The barks of *V. copallifera* and *S. stipularis* are used in Sri Lanka to prevent fermentation of toddy in the local jaggery industry. These two dipterocarps contain the same two polyphenols. Copalliferol A (2) inhibited the growth of *Oxford staphylococcus* and *Escherichia coli* in the Mueller Hinton Agar (MHA) medium tested by the filter paper disc method.

The question, whether the two polyphenols which are trimers of resveratrol are phytoalexins or natural products, was settled by immediate extraction of the dried milled bark with cold acetone. The yields of the polyphenols remained as indicated in the Table showing that these polyphenols are indeed natural products.

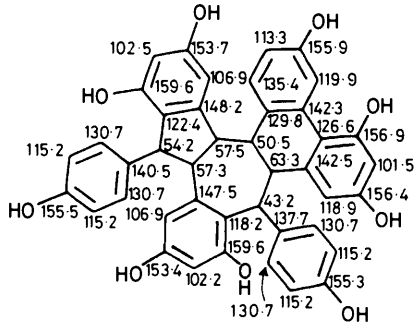
## Experimental

The barks of *Vateria copallifera* and *Shorea stipularis* were collected from the Kanneliya forest in the South of Sri Lanka, the bark of *Balanocarpus zeylanicus* (=*Hopea brevipetiolaris*) was collected from Arankele near Kurunegala in the central hills of Sri Lanka and the bark of *Hopea cordifolia* was collected on the Kirindi Oya bank in the South of Sri Lanka. For all other details see earlier paper.[6]

The powdered bark, in each case, was extracted with cold acetone. Evaporation of the solvent gave a brown powder which was chromatographed on a column of silica gel. Elution with benzene removed the oily hydrocarbons and terpenes. Polyphenols were eluted with acetone–benzene mixtures. P.t.l.c. purification of the column fractions (see ref. 6 for details) gave t.l.c. pure polyphenols.




**Scheme 2.** Mass spectral fragments of copalliferol A



**Scheme 3.** $^{13}C$ N.m.r. chemical shift assignments of copalliferol A.

*Stemonoporol* (3).—Elution of the column with acetone–benzene (3 : 7) gave stemonoporol (3) as a pale brown solid which was purified by p.t.l.c. to yield a pale white amorphous solid, m.p. >300 °C (decomp.) (Found: $M^+$ 680.1979, $C_{42}H_{32}O_9$ requires $M^+$ 680.2046); $\lambda_{max.}$ (MeOH) 283 nm (ε 2 550), $\lambda_{max.}$ (MeOH/NaOH) 296 nm (ε 4 250); no shift with NaOAc–$H_3BO_3$; $\nu_{max.}$ (KBr) 3 250, 1 600 and 830 $cm^{-1}$; $\delta_H$ [$(CD_3)_2CO$, 360 MHz) 4.30 (1 H, dd), 4.51 (2 H, m), 4.84 (1 H, d), 5.17 (1 H, s), 6.04—7.57 (alkene and aromatic protons); *m/z* 680 ($M^+$ 12%), 678 (13), 662 (12), 588 (10), 587 (18), 586 (34), 574 (8), 573 (13), 572 (15), 566 (13), 495 (20), 494 (49), 493 (7), 492 (12), 482 (21), 480 (8), 466 (10), 450 (15), 435 (11), 434 (36), 200 (6), 107 (18), 95 (10), and 94 (100).

*Nonamethylstemonoporol.*—Stemonoporol (200 mg) was refluxed with $Me_2SO_4$ (0.7 ml) and anhydrous $K_2CO_3$ (800 mg) in anhydrous acetone (30 ml) for 36 h. The usual work-up gave the product, m.p. 138—140 °C which was identical with an authentic sample [5] (t.l.c., i.r., $^1H$ n.m.r., mixed m.p.).

**Table**

| Dipterocarp species | Polyphenols present (%) [a] Copalliferol A(2) | stemonoporol (3) |
|---|---|---|
| *Vateria copallifera* | 0.98 | 1.29 |
| *Balanocarpus zeylanicus* (= *Hopea brevipetiolaris*) | 0.18 | |
| *Hopea cordifolia* | 0.09 | |
| *Shorea stipularis* | 0.05 | 0.08 |

[a] Percentage expressed with respect to the dry weight of plant material.

*Nona-acetate of stemonoporol.*—Stemonoporol (200 mg) was treated with acetic anhydride (1.0 ml) and pyridine (4 ml). The reaction mixture was initially warmed and was kept at room temperature for 24 h. The acetate was isolated and was purified by p.t.l.c. to give the nona-acetate, m.p. 166—168 °C. It was found to be identical with an authentic sample [5] t.l.c., i.r., $^1H$ n.m.r., mixed m.p.).

*Copalliferol A* (2).—When the column containing this polyphenol was eluted with acetone–benzene (7 : 13) copalliferol A (2) separated as an off-white amorphous solid. This was purified by p.t.l.c. to give the pure polyphenol, m.p. >300 °C (decomp.), $[\alpha]_D^{25}$ +115.6° (MeOH) (Found: $M^+$ 680.2075, $C_{42}H_{32}O_9$ requires *M*, 680.2046); $\lambda_{max.}$ (EtOH) 282 nm (ε 867), $\lambda_{max.}$ EtOH/NaOH 288 nm (ε 1 054), $\lambda_{max.}$ (EtOH/NaOMe) 291 nm (ε 1 207); no shifts with NaOAc/$H_3BO_3$; $\nu_{max.}$ (KBr) 3 250, 1 600, 1 500, 1 440, 1 330, 1 100, 1 010, 830 $cm^{-1}$; $\delta_H$[$(CD_3)_2CO$, 360 MHz), 3.51 (1 H, d, *J* 6.75 Hz), 3.86 (1 H, dd, *J* 6.75 and 2.0 Hz), 4.10 (1 H, s), 4.55 (1 H, d, *J* 3.75 Hz), 4.63 (1 H, d, *J* 3.73 Hz), 4.90 (1 H, d, *J* 2.0 Hz), 5.67 (1 H, d, *J* 2 Hz), 5.76 (1 H, dd, *J* 2.0 and 4.0 Hz), 5.97 (5 H, d, *J* 2.0 Hz), 6.03 (1 H, d, *J* 4 Hz), 6.11 (2 H, dd, *J* 2.0 and 7.0

Hz), 6.22 (1 H, s, *J* 2 Hz), 6.48 (6 H, dd, *J* 2.0 and 4.0 Hz), 6.70 (br, OH), 7.14 (br, OH), 7.52 (s, OH), 7.97 (s, OH), 8.05 (s, OH), and 8.07 (s, OH); δ[$(CD_3)_2CO$], $^{13}C$ n.m.r. off-resonance proton decoupled spectrum) see Scheme 3; *m/z* 680 ($M^+$, 13%), 482 (40), 466 (33), 464 (35), 398 (42), 360 (56), 359 (61), 350 (70), 348 (72), 332 (69), 330 (65), 243 (44), 226 (39), 216 (50), 200 (100), 199 (90), 183 (72), 181 (70), 107 (95), 77 (66), 66 (68), and 65 (75)

*Nonamethyl Ether of Copalliferol A* —Copalliferol A (150 mg) was refluxed with dimethyl sulphate (0.6 ml) and anhydrous $K_2CO_3$ (500 mg) in anhydrous acetone (30 ml) for 24 h The usual work-up gave a crude product which was separated by p.t.l.c. to give the pure nonamethyl ether, m.p. 145—147 °C, $[\alpha]_D^{25}$ +92.8° ($CHCl_3$) $M^+$ 806.3600, $C_{51}H_{50}O_9$ requires $M^+$ 806.3606; $\delta_H$ ($CDCl_3$, 60 MHz) 3.46 (s, 2 × $OCH_3$), 3.55 (d, *J* 4 Hz), 3.66 (s, $OCH_3$), 3.70 (s, 2 × $OCH_3$), 3.73 (s, $OCH_3$), 3.86 (dd, *J* 2.0 and 4.0 Hz), 4.10 (s, $OCH_3$), 4.27 (s), 4.6 (dd, *J* 3.0 and 6.0 Hz), 4.86 (d, *J* 3 Hz), 5.66 (d, *J* 2 Hz), 5.78 (d, *J* 2.0 Hz), 6.0 (s), 6.1—6.60 (m, aromatic protons); *m/z* 806 ($M^+$, 100%), 774 (34), 579 (56), 566 (48), 565 (70), 550 (16), 459 (48), 447 (42), 441 (26), 429 (44), 418 (20), 388 (44), 268 (28), 257 (23), 239 (46), 227 (40), and 121 (62).

*Nona-acetate of Copalliferol A*.—The acetate of copalliferol A (200 mg) was prepared by treatment of the polyphenol with acetic anhydride (1.0 ml) and pyridine (5.0 ml). The mixture was warmed and was kept at room temperature for 24 h. After work-up, the crude product obtained was purified by p.t.l.c. to give the pure acetate, m.p. 176—178 °C; $\delta_H$ ($CDCl_3$, 60 MHz), 1.93 (s, $OCOCH_3$), 2.06 (s, $OCOCH_3$), 2.13 (s, $OCOCH_3$), 2.20 (s, 3 × $OCOCH_3$), 2.23 (s, $OCOCH_3$), 2.33 (s, $OCOCH_3$), 2.53 (s, $OCOCH_3$), 3.66 (d, *J* 2.0 Hz), 3.80 (m), 4.16 (br), 4.43 (dd, *J* 2.0 and 6.0 Hz), 4.70 (dd, *J* 2.0 Hz and 4.0 Hz), 5.8—7.2 (overlapping multiplets, aromatic protons).

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## References

1 P. Coggon, N. F. Janes, F. E. King, T. J. King, R. J. Molyneux, J. W. W. Morgan and K. Sellars, *J. Chem. Soc.*, 1965, 406.
2 R. Madhav, T. R. Sechadri, and G. B. V. Subramaniam, *Phytochemistry*, 1967, **61**, 1155.
3 R. J. Pryce and P. Langcake, *Phytochemistry*, 1977, **16**, 1452.
4 M. U. S. Sultanbawa, S. Surendrakumar, and P. .Bladon, *J. Chem. Soc., Chem. Commun.*, 1980, 619.
5 U. Samaraweera, S. Sotheeswaran, and M. U. S. Sultanbawa, *Phytochemistry*, 1982, **21**, 2585.
6 S. P. Gunasekera, S. Sotheeswaran, and M. U. S. Sultanbawa, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1833.

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