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DISTICHOL, AN ANTIBACTERIAL POLYPHENOL FROM *SHOREA DISTICHA*

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Key Word Index *Shorea disticha*; Dipterocarpaceae; bark; distichol; antibacterial activity.

Abstract—The structure of distichol, a new hexadehydrotrimer of resveratrol isolated from the bark of *Shorea disticha*, has been established on the basis of spectroscopic evidence, chemical degradations and biosynthetic considerations.

INTRODUCTION

Several oligomeric stilbenols have been reported from the plant family Dipterocarpaceae [1–5], of which hopeaphenol (1) [6, 7] was the first, and its structure was established as a tetramer of 3,5,4′-trihydroxystilbene (resveratrol) by X-ray analysis [8]. In our continuing study of these polyphenols, we have isolated distichol (2) [9], a new hexadehydrotrimer of resveratrol, frm the bark of *Shorea disticha* (Thw.) Ashton. The plant also contains the dehydrodimer, ε-viniferin (3) reported previously from infected grapevine leaves (*Vitis vinifera*) [10] and *Vatica affinis* [3, 4].

RESULTS AND DISCUSSION

The cold acetone extracts of the bark, after chromatographic purification afforded distichol (2), [M]$^+$ 680.2027 ($C_{42}H_{32}O_9$). Its UV absorption λ_{max}^{MeOH} 281 (log ε 4.21) nm] was virtually the same as those of related polyphenols, and the spectrum was unaffected on addition of sodium acetate–boric acid which excluded the presence of *ortho* dihydric phenol structures. The IR (KBr) spectrum showed a broad band at 3200 cm^{-1} (OH), aromatic absorption at 1600 cm^{-1} and a prominent band at 830 cm^{-1} indicative of 1:4 disubstituted benzene nuclei.

Distichol (2) formed an octamethylether, [M]$^+$ 792.3310 ($C_{50}H_{48}O_9$) and an octaacetate. Therefore the remaining one oxygen atom was probably present as an ether group. The ^{13}C NMR spectrum (acetone-d_6) of the parent compound (2) showed the following resonances: six doublets (δ_C 37–91 ppm) due to six aliphatic carbon atoms, six singlets (δ_C 155–161) for nine phenolic carbon atoms, 10 doublets (δ_C 97–131) assigned to a total of 18 aromatic carbon atoms, and nine singlets (δ_C 121–147) for nine quaternary carbon atoms. (The designations singlets and doublets refer to the appearance in single frequencey off-resonance proton decoupled experiments.) The high frequency ^{1}H NMR of distichol and its derivatives showed clear resonances due to the presence of 18 aromatic protons and six coupled methine protons (δ_H 3.60–5.85 ppm).

The above data and the molecular formula of distichol ($C_{42}H_{32}O_9$) showed that the polyphenol is probably formed by trimerization of resveratrol units ($C_{14}H_{12}O_3$). The absence of any olefinic groups in distichol is evident from both the ^{1}H and ^{13}C NMR spectral features: the ^{13}C NMR of distichol reveals six doublets for six aliphatic methine carbons, thus suggesting that all six olefinic carbons corresponding to the three resveratrol units are saturated and carry single protons. These six methine protons clearly showed resonances in the ^{1}H NMR spectrum. Extensive homonuclear decoupling experiments of the ^{1}H NMR spectrum indicated that the methine protons at δ5.84 (H_1) and δ4.47 (H_2) are coupled to one another and the remaining four coupled protons are adjacent to each other (see partial structure **2a**). The ^{1}H NMR spectrum of distichol showed resonances due to the presence of 18 aromatic protons out of a total of 21 aromatic protons from the three resveratrol units. This suggests that phenolic oxidative coupling has occurred at three aromatic positions. Thus it is probable that biogenetically distichol (2) is formed from the dehydrodimer of resveratrol (3) and a free resveratrol moiety (Scheme 1). The isolation of the dehydrodimer of resveratrol (ε-viniferin) from the same extracts lends further support to the structure (2) assigned to distichol. The other biogenetically plausible structures were eliminated because of the absence of any unsaturated double bonds in the molecule. This narrows the possibilities to two, one of which has a nine-membered ring. However, this possibility does not satisfactorily explain the spectral behaviour.

Further evidence for the structure (2) of distichol included the formation of the following chemical degradative products: picric acid obtained by oxidation of 2 with concentrated nitric acid; 3-nitroanisic acid obtained by oxidation of the octamethyl ether of 2 with nitric acid; anisic acid obtained by oxidation of octamethyl ether of 2 with CrO_3–HOAc; and 4-hydroxybenzoic acid obtained by alkali fusion of 2 with NaOH–KOH (1:1) at 270°. (All these products were characterized by mp, mmp and comparison with authentic samples.)

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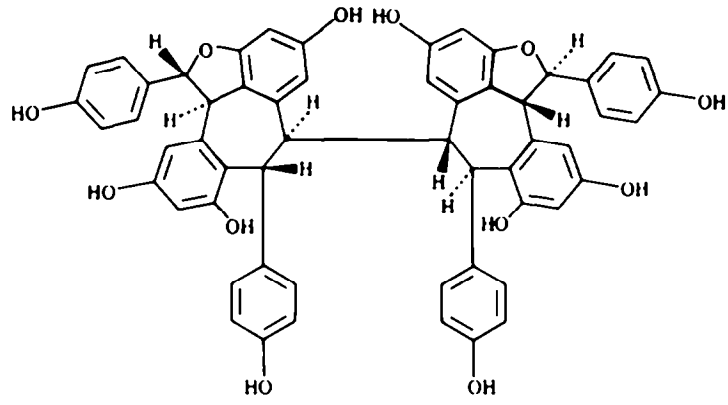

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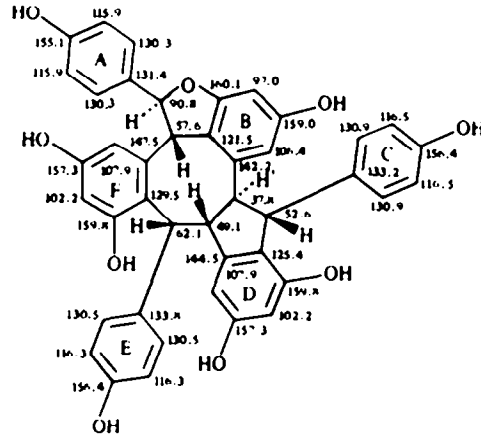

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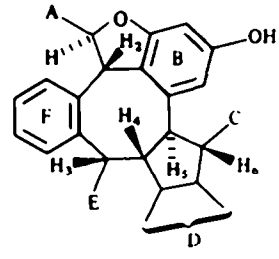
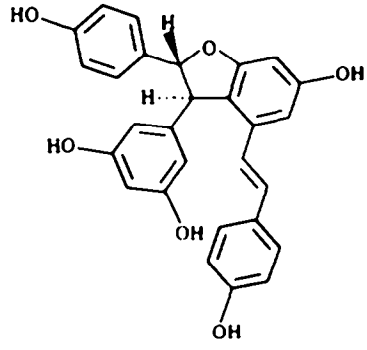


2a



3

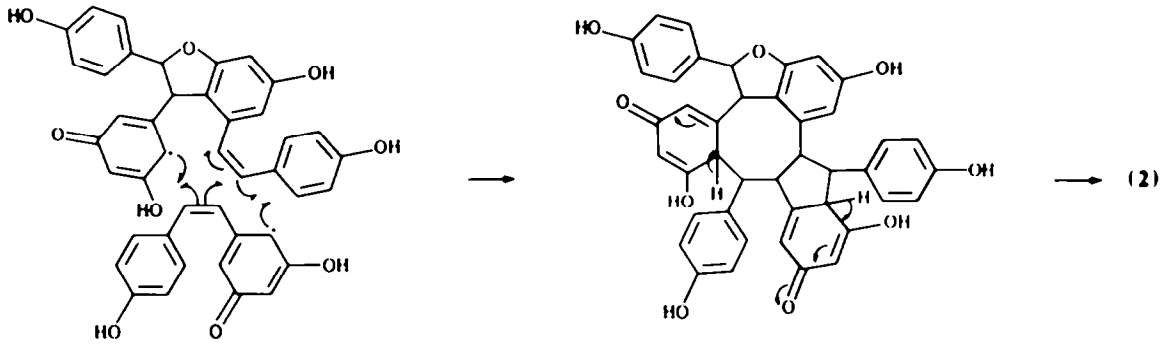
The proposed stereochemistry for distichol was chosen by comparison of a Dreiding model with the observed coupling constants (confirmed by decoupling experiments). The eight-membered ring is in the boat conformation, in agreement with the unique coupling 'through space' observed between the protons δ4.47 (H_2) and δ3.65 (H_4) (**2a**).

Distichol octamethyl ether gives characteristic odd electron fragmentations in the mass spectrometer at *m/z* 684 (84%), 538 (55) and 430 (24) due to cleavage of phenolmethyl ethers, benzylic methyl ethers and resveratrol moieties.

Both distichol (**2**) and ε-viniferin (**3**) isolated in this study showed antibacterial activity towards Oxford *Staphylococcus* and *Escherichia coli* when tested by the filter paper disc method in Mueller Hinton Agar medium. Resveratrol oligomers from *Gnetum* species have been studied by Lins *et al.* [11]. Probable assignments of ^{13}C NMR chemical shifts based on substituent parameters are shown in structure **2**.

EXPERIMENTAL

Dried, powdered bark (3.30 kg) of *S. disticha* collected in the Kanneliya rain forest in the South of Sri Lanka was exhaustively extracted with cold Me_2CO in the dark for 7 days. The filtrate was concd under reduced pressure to give a pale brown powder (275 g, 8.30%). The extract (30.0 g) was transferred to a column of



Scheme 1.

silica gel (Merck, 30–70 mesh) and eluted successively with C_6H_6–Me_2CO solvent mixtures for increasing polarity. The Me_2CO–C_6H_6 (3:7) eluates yielded **3**, mp 147–149° (191 mg) identified as ε-viniferin by comparison with the authentic sample previously isolated [3, 4].

Isolation of distichol (**2**). Elution with Me_2CO–C_6H_6 (2:3) afforded distichol as an off-white solid foam (5.68 g). This was further purified by prep. TLC (silica gel; PF 254) to give **2** as an amorphous solid, mp 266–268°, $[\alpha]_D^{25}$ −44° (MeOH). M⁺ 680.2027 ($C_{42}H_{32}O_9$) ($C_{42}H_{32}O_9$ requires M 680.2046). UV λ_{max}^{MeOH} nm (log ε); 281 (4.21). No shifts with NaOAC H_3BO_3; IR ν_{max}^{KBr} cm⁻¹: 3200, 1600, 1440, 1330, 1220, 1140, 1070, 1000, 920 and 830; ^{1}H NMR (360 MHz, Me_2CO-d_6): 3.65 (1H, *dd*, *J* = 3.2 and 11.7 Hz, H-4), 3.76 (1H, *dd*, *J* = 11.7 and 9.5 Hz, H-5), 4.25 (1H, *d*, *J* = 9.5 Hz, H-6) 4.47 (1H, *d*, *J* = 11.7 Hz, H-2), 5.28 (1H, *d*, *J* = 3.2 Hz, H-3), 5.84 (1H, *d*, *J* = 11.7 Hz, H-1) and 6.15–7.35 (overlapping multiplets, 18 Ar-Hs); ^{13}C NMR: see **2**; MS *m/z* (rel. int.): 680 [M]⁺ (35), 678 (29), 662 (25), 588 (29), 586 (52), 568 (16), 566 (15), 494 (39), 492 (27), 490 (13), 482 (47), 481 (16), 451 (16), 450 (47), 435 (40), 434 (68), 422 (33), 390 (47), 389 (13), 359 (17), 228 (48), 212 (36), 200 (36), 199 (20), 108 (36), 107 (56), 95 (45), 94 (100), 66 (68), 65 (64), 63 (32). When refluxed (36 hr) with Me_2SO_4 (1.0 ml), K_2CO_3 (1.5 g) and dry Me_2CO (25 ml) **2** (500 mg) gave an octamethyl ether, amorphous solid, mp 138–140° (496 mg; 85.2%), $[\alpha]_D^{25}$ −49° ($CHCl_3$). M⁺ 792.3310 ($C_{50}H_{48}O_9$) ($C_{50}H_{48}O_9$ requires M 792.3298); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 283 (4.04); IR ν_{max}^{KBr} cm⁻¹: 2910, 2810, 1600, 1505, 1455, 1310 (*br*), 1245, 1195, 1170, 1150, 1060, 1030, 920, 830 and 690; ^{1}H NMR (270 MHz, $CDCl_3$): 3.53 (3H, *s*, OMe), 3.60 (3H, *s*, OMe), 3.70 (9H, *s*, 3 × OMe), 3.73 (6H, *s*, 2 × OMe), 3.80 (3H, *s*, OMe), 4.33 (1H, *d*, *J* = 10 Hz), 4.58 (1H, *d*, *J* = 6 Hz), 4.87 (*m*), 5.27 (1H, *d*, *J* = 2 Hz), 5.50 (1H, *d*, *J* = 10 Hz), 6.0–7.45 (m); MS, *m/z* (rel. int.): 792 [M]⁺ (65) 790 (48), 684 (100), 671 (17), 669 (16), 552 (35), 539 (32), 538 (55), 522 (35), 430 (24), 415 (15), 396 (19), 387 (24), 343 (43), 342 (59), 288 (49), 281 (20), 273 (37), 257 (9), 227 (32), 121 (36). **2** (200 mg) gave an octaacetate when treated with Ac_2O (1.0 ml) and C_5H_5N (3.0 ml) at room temp. (24 hr) solid with a glassy appearance, mp 162–164°, (180 mg, 61%), $[\alpha]_D^{25}$ −15.2° (MeOH); IR ν_{max}^{KBr} cm⁻¹: 2905, 1750, 1640, 1600, 1500, 1450 (*br*), 1365, 1180, 1120, 1070, 1020, 910, 840 and 670; ^{1}H NMR (270 MHz, $CDCl_3$): 1.66 (*s*, OAc), 1.83 (*s*, OAc), 2.23 (*s*, 2 × OAc), 2.26 (*s*, 4 × OAc), 3.90 (*dd*, 1H, *J* = 8 and 4 Hz), 4.26 (1H, *m*), 4.36 (*d*, 1H, *J* = 8 Hz), 4.43 (*d*, 1H, *J* = 11 Hz), 4.73 (1H, *m*), 5.90 (*d*, 1H, *J* = 11 Hz), 6.46–7.46 (*m*).

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