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CANALICULATOL, AN ANTIFUNGAL RESVERATROL TRIMER FROM *STEMONOPOROUS CANALICULATUS*

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Key Word Index—*Stemonoporus*; polyphenol; canaliculatol; antifungal.

Abstract—A new resveratrol trimer, canaliculatol, has been isolated from the bark of *Stemonoporus canaliculatus*. Canaliculatol showed antifungal activity against the fungus, *Cladosporium cladosporioides*. The structure and stereochemistry of canaliculatol is reported in this paper.

INTRODUCTION

Polyphenols from six *Stemonoporus* species were reported in ref. [1]. The investigation of *S. affinis*, *S. cordifolius*, *S. elegans*, *S. kanneliensis*, *S. lancifolius* and *S. oblongifolius* revealed [1] the presence of the following polyphenols: bergenin (**1**), stemonoporol (**2**), copalliferol A (**3**) and vaticaffinol (**4**). The structure of vaticaffinol has been revised [2] as **5**. In this paper, we report the identification of yet another new polyphenol, named canaliculatol, from the bark extractives of *S. canaliculatus*.

RESULTS AND DISCUSSION

The acetone extract of the bark of *S. canaliculatus* showed antifungal activity against the fungus *Cladosporium cladosporioides*. The column chromatographic separations on silica gel gave a mixture of two major polyphenols. These were separated by preparative TLC to give a polyphenol which was identical with vaticaffinol isolated earlier [1, 2]. The second major polyphenol showed antifungal activity against *C. cladosporioides*. It was found to be a new polyphenol and the mass spectral data showed (M^+, *m/z* 680) it to be resveratrol (**6**) trimer. It has been named canaliculatol. The ^{1}H NMR spectrum of canaliculatol showed the presence of: (i) 12 aromatic protons each showing *ortho* coupling at δ7.16 (2H, *d*, J = 7.0 Hz), 7.12 (2H, *d*, J = 7.0 Hz), 6.95 (2H, *d*, J = 8.6 Hz), 6.73 (2H, *d*, J = 8.6 Hz), 6.68 (2H, *d*, J = 8.5 Hz) and 6.63 (2H, *d*, J = 8.7 Hz); (ii) six aromatic protons each showing *meta* coupling at δ6.21 (1H, *d*, J = 2.2 Hz), 6.18 (3H, *m*), 5.98 (1H, *d*, J = 2.2 Hz), 5.16 (1H, *d*, J = 2.6 Hz); and (iii) six aliphatic protons at δ5.75 (1H, *d*, J = 11.7 Hz), 4.63 (1H, *br s*), 4.30 (1H, *d*, J = 11.7 Hz), 4.17 (1H, *d*, J = 8.4 Hz), 3.67 (1H, *d*, J = 8.4 Hz) and 3.65 (1H, *br s*). These results show that, like the other polyphenols isolated [1] from this plant genus, canaliculatol is made up of three resveratrol units.

The ^{13}C NMR spectrum of canaliculatol showed the presence of six aliphatic carbon atoms of the CH type at δ37.57, 52.52, 52.52, 58.14, 62.62 and 90.99. The resveratrol trimer, copalliferol A, on the other hand, had [3] its aliphatic carbons of the CH type resonating in its ^{13}C NMR spectrum at δ43.2, 50.5, 54.2, 57.3, 57.5 and 63.3. The presence of the signal at δ90.99 in the ^{13}C NMR spectrum of canaliculatol indicated that, unlike copalliferol A, canaliculatol had a dihydrofuran ring found in the resveratrol dimers, balanocarpol [4] and viniferin [5], in the resveratrol trimer, distichol [6] and in the resveratrol tetramer, vaticaffinol [5].

Canaliculatol on complete acetylation gave an octaacetate and methylation with dimethyl sulphate gave an octamethyl ether, M^+, *m/z* 792. These results show that canaliculatol has eight free hydroxyl groups. These data confirm that canaliculatol is derived from three resveratrol ($C_{14}H_{12}O_3$) units and show that out of the nine oxygen atoms in canaliculatol, eight are present as hydroxyl functions and one is present in a dihydrofuran ring.

The ^{1}H NMR data of the octamethyl ether of canliculatol confirmed the presence of 12 *ortho*-coupled aromatic protons, six *meta*-coupled aromatic protons and six aliphatic ring protons in the skeleton of canaliculatol. These data are summarised below: *ortho*-coupled aromatic protons: δ7.33 (2H, *d*, J = 8.7 Hz), 7.16 (2H, *d*, J = 8.5 Hz), 7.02 (2H, *d*, J = 8.7 Hz), 6.86 (2H, *d*, J = 8.7 Hz), 6.79 (2H, *d*, J = 8.7 Hz), 6.81 (2H, *d*, J = 8.8 Hz). *meta*-coupled aromatic protons: δ6.40 (1H, *d*, J = 2.3 Hz), 6.32 (1H, *s*), 6.28 (1H, *d*, J = 2.3 Hz), 6.23 (2H, *d*, J = 2.3 Hz), 6.19 (1H, *d*, J = 2.3 Hz). Aliphatic ring protons: δ5.89 (1H, *d*, J = 11.9 Hz), 5.26 (1H, *d*, J = 2.4 Hz), 4.5 (1H, *d*, J = 11.9 Hz), 4.28 (1H, *dd*, J = 2.3 and 10.9 Hz) and 3.88 (2H, *m*).

The ^{13}C NMR data of the octamethyl ether of canaliculatol showed the presence of: (i) six aliphatic ring carbon atoms of the CH type at δ36.65, 47.73, 50.91, 57.47, 60.91 and 90.16; (ii) aromatic carbons atom of the CH type at δ93.18, 97.30, 98.22, 104.41, 105.75, 105.80, 113.34, 113.49, 114.18, 129.18, 129.32 and 129.68 ppm, the signals at δ113.34, 113.49, 114.18, 129.18, 129.32 and 129.68 integrated for two carbon atoms each showing a C^2 symmetry of the *para*-substituted aromatic ring [4]; (iii)

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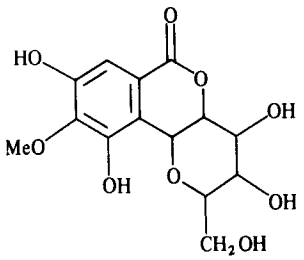

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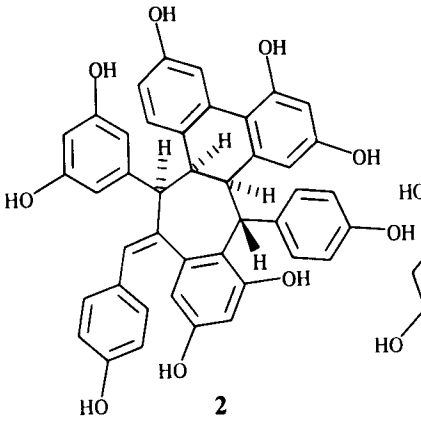

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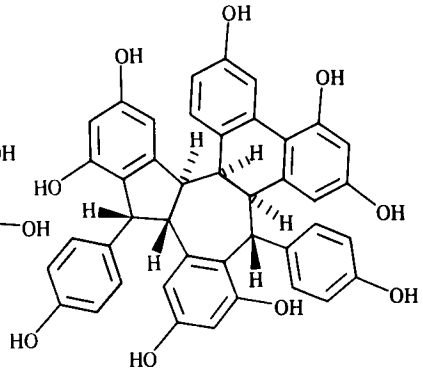

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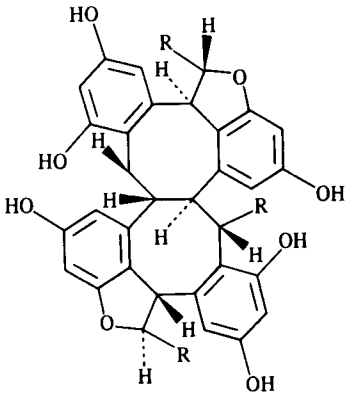
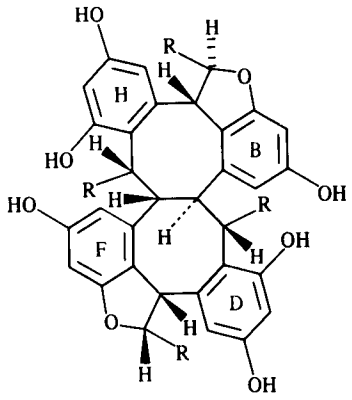
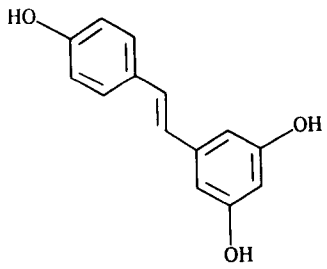


4 R = C_6H_4OH-*p*



5 R = PhOH-*p*



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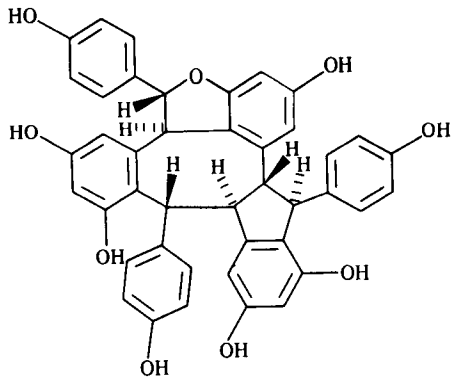

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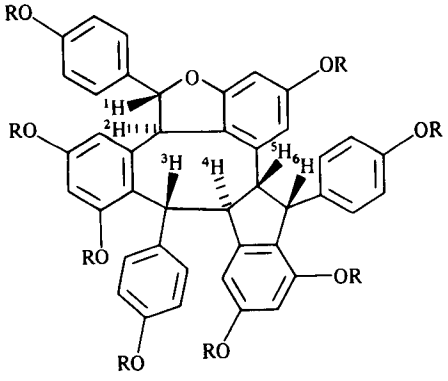


9 R = H canaliculatol
R = Me canaliculatol octamethylether
R = COMe canaliculatol octaacetate

nine aromatic quaternary carbon atoms each attached to an oxygen atom at δ160.17, 160.11, 160.01, 158.63, 158.55, 158.22, 157.70, 157.33, 157.07 and (iv) nine other aromatic quaternary carbon atoms at δ146.62, 145.61, 143.21, 140.44, 133.00, 129.70, 127.30, 121.95 and 115.21.

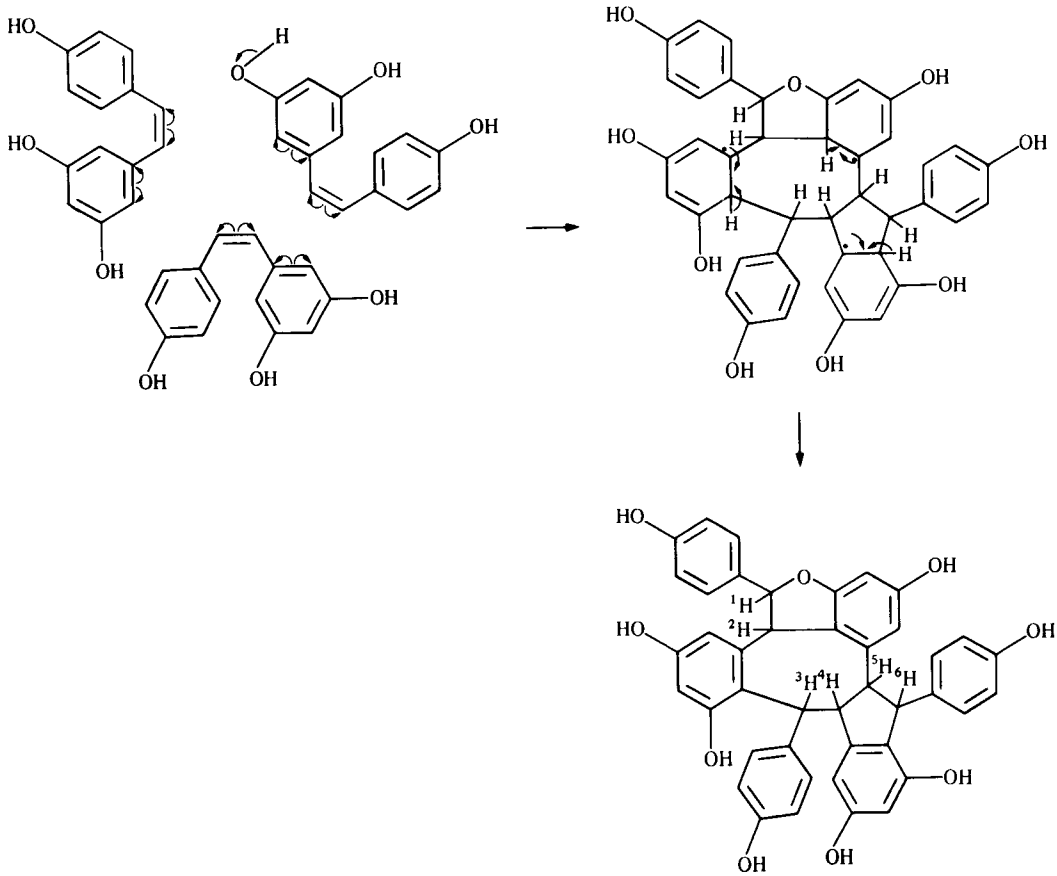
Based on the above data, the structure of canaliculatol can be written as **8** and its biosynthesis from three units of resveratrol (**6**) can be represented as in Fig. 1. Canaliculatol (**8**) and distichol (**7**) [6], thus, have the same structural formula but are not identical since the physical properties of the phenols and their derivatives were different (see Table 1). Canaliculatol should, therefore, be a stereoisomer of distichol (**7**).

The ^{1}H NMR chemical shifts of the aliphatic ring protons of canaliculatol and its derivatives have been assigned as shown below. The stereochemistry of the aliphatic ring protons of canaliculatol was obtained from the coupling constant data and NOE experiments. These results are summarised below. The proton numbering refers to structure (**8**). Canaliculatol: δ5.75 (1H, *d*, J = 11.7 Hz, H-1), 4.30 (1H, *d*, J = 11.7 Hz, H-2), 4.17 (1H, *d*, J = 8.4 Hz, H-3), 3.67 (1H, *d*, J = 8.4 Hz, H-4), 3.65 (1H, *br s*, H-5) and 4.63 (1H, *br s*, H-6). Homodecoupling experiments showed that the protons at δ5.75 and 4.30 were coupled to each other. The coupling constant values showed these protons (H-1, H-2) to be *trans* oriented. The homodecoupling experiments also indicated that the proton at δ4.17 (H-3) was coupled to one of the protons at δ3.67 (H-4).

Table 1. Physical properties of canaliculatol (**8**) and distichol (**7**)

	Mp	$[\alpha]_D$
Canaliculatol	245° (dec.)	−25.5 (MeOH)
Distinchol [6]	266–268°	−44.2 (MeOH)
Canaliculatol octamethyl ether	133–136°	−34.0 ($CHCl_3$)
Distichol octamethyl ether [6]	138–140°	−48.9 ($CHCl_3$)
Canaliculatol octaacetate	158–161°	−75.0 ($CHCl_3$)
Distichol octaacetate [6]	142–164°	−15.2 ($CHCl_3$)

Canaliculatol octamethylether: δ5.89 (1H, *d*, J = 11.95 Hz, H-1), 4.50 (1H, *d*, J = 11.9 Hz, H-2), 4.28 (1H, *dd*, J = 2.3 and 10.9 Hz, H-3), 3.88 (2H, *m*, H-4 and H-5) and 5.26 (1H, *d*, J = 2.3 Hz, H-6). Homodecoupling of the proton at δ5.89 resulted in the doublet at δ4.5 becoming a singlet and homodecoupling of the proton at δ4.5 enabled the doublet at δ5.89 to collapse to a singlet. Irradiation of the protons at δ5.28 and at 4.28 resulted in the signal at δ3.88 changing its multiplicity. These data confirm that the protons at δ5.89 (H-1) and at δ4.50 (H-2) are *trans* coupled and that the protons at δ5.26 (H-6) and at δ4.28



8

Fig. 1. Biosynthesis of compound **8**.

(H-3) are coupled to the two protons (H-4) and H-5) which appeared under the –OMe signals at 3.88.

Canaliculatol octaacetate: δ5.97 (1H, *d*, J = 11.8 Hz, H-1), 4.34 (1H, *d*, J = 11.8 Hz, H-2), 4.31 (a proton signal under the doublet, H-3), 3.88 (1H, *d*, J = 10.8 Hz, H-4), 3.93 (1H, *br s*, H-5) and 4.69 (1H, *br s*, H-6). Homodecoupling experiments showed that the protons at δ5.97 (H-1) and at δ4.34 (H-2) are coupled to each other. Irradiation of the proton at δ3.88 resulted in the proton at δ4.31 becoming a singlet. Irradiation of the proton at δ4.31 made the signal at δ3.88 collapse to a singlet. Irradiation of the proton at δ4.69 produced no change in the signals. The NOE difference experiments on the acetate gave valuable information regarding the relative stereochemistry of the aliphatic ring protons. Saturation of the signal at δ4.69 led to a strong enhancement of the signal at δ3.93 confirming the *cis* orientation of the protons H-5 and H-6. Saturation of the proton at δ5.97 showed NOE s of the proton signals at δ4.69, 4.31 and 3.93. This shows that the relative configurations of the protons H-1, H-6, H-3 and H-5 are *cis*.

The relative stereochemistry of the aliphatic ring protons of canaliculatol based on the above data is shown in structure **9**.

EXPERIMENTAL

The bark of *Stemonoporus canaliculatus* was collected from the Kanneliya forest in the south of Sri Lanka. Bark (3 kg) was successively extracted with petrol and cold Me_2CO. The Me_2CO extract (150 g) contained the polyphenols.

The Me_2CO extract was subjected to CC on silica gel which was eluted first with C_6H_6 to remove less polar materials. Elution with C_6H_6–Me_2CO (1:1) gave the crude polyphenol mixture (5 g). The major polyphenol was found to be vaticaffinol [1]. The minor polyphenol was purified by prep. TLC to give analytically pure canaliculatol.

MP: uncorr; ^{1}H NMR and ^{13}C NMR: 250 MHz $[\alpha]_D^{25}$: 3 mg/10 ml.

Canaliculatol. Mp 245° (dec.) $[\alpha]_D$ – 25.5° (MeOH), yield (0.15%); uv λ_{max} 282 nm log ε 4.21; IR ν_{max} (KBr): 3200 (OH), 1600 (C=C), and 830 cm^{-1}; MS *m/z* 680 $[M^-]^+$ 100%), 586 (30), 573 (25), 554 (5), 482 (60), 464 (30); ^{1}H NMR (CD_3OD): see text.

Canaliculatol octamethylether. Canaliculatol (150 mg) was refluxed with Me_2SO_4 (0.5 ml) in Me_2CO (15 ml) and K_2CO_3 (500 mg) for 24 hr. The methyl ether was worked-up in the usual way to give the crude product which was purified by prep. TLC to give pure methyl ether (60 mg), mp 133–36°, $[\alpha]_D$ – 34° ($CHCl_3$); ^{1}H NMR ($CDCl_3$): δaromatic and aliphatic protons (see text), 3.79 (3H, *s*, OMe), 3.78 (3H, *s*, OMe), 3.74 (3H, *s*, OMe), 3.69 (9H, *s*, 3 × OMe), 3.66 (3H, *s*, OMe), and 3.55 (3H, *s*, OMe); ^{13}C NMR ($CDCl_3$): see text.

Canaliculatol octaacetate. Canaliculatol (150 mg) was acetylated using Ac_2O (1.0 ml) and pyridine (1 ml) at room temp. for 24 hr. After work-up and purification by prep. TLC, analytically pure acetate (75 mg) was obtained, mp 158–61°, $[\alpha]_D$ – 75° ($CHCl_3$); ^{1}H NMR ($CDCl_3$): δ6.4–7.4 (24 H, aromatic protons), 3.88–5.97 (aliphatic ring protons) (see text), 2.29 (9H, *s*, 3 × OCOMe), 2.28 (6H, *s*, 2 × OCOMe), 2.24 (3H, *s*, OCOMe), 1.84 (3H, *s*, OCOMe), 1.65 (3H, *s*, OCOMe).

TLC-Bioassay of polyphenol for anti-fungal activity. The polyphenol was subjected to TLC (silica gel), MeOH–CH_3Cl_2, 17:83). The plate was dried in air overnight, sprayed with *Cladosporium cladosporioides* in Czapex-Dox nutrient soln and incubated in a moist chamber at room temp. for 48 hr. The region in which the fungal growth was inhibited, appeared light coloured against the mycelium background.

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