The Metabolites of *Trichoderma longibrachiatum*. III*† Two New Tetronic Acids: 5-Hydroxyvertinolide and Bislongiquinolide

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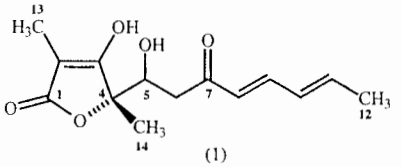
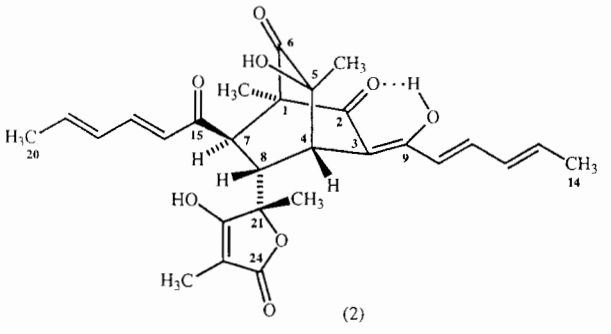
Two new tetronic acid derivatives, 5-hydroxyvertinolide (1) and bislongiquinolide (2), have been isolated from the fungus *Trichoderma longibrachiatum* Rifai aggr., which is antagonistic to the fungus *Mycena citricolor*, the causative agent of the American leaf spot disease of coffee. The structures were determined by a combination of spectroscopic techniques, including ^{1}H and ^{13}C n.m.r., i.r., u.v., c.d., and mass spectrometry.

In Parts I and II of this series we reported the structures of trichodimerol, trichodermolide, and sorbiquinol, produced by *Trichoderma longibrachiatum* Rifai aggr., a fungus antagonistic to *Mycena citricolor*, the causative agent of the American leaf spot disease of coffee.[1,2] In this article we describe the structure elucidation of two new tetronic acid derivatives produced by this fungus: 5-hydroxyvertinoide (1) and bislongiquinolide (2).



(1)



(2)

5-Hydroxyvertinolide was obtained as a colourless solid, $[\alpha]_D$ −64° (MeOH). The u.v. spectrum shows absorptions at 230sh, 263 and 359 nm, which are shifted upon addition of base, indicating that the chromophore is acidic. The i.r. spectrum shows absorptions for hydroxy groups (3360 cm^{-1}), carbonyl groups (1749, 1666 cm^{-1}) and double bonds (1638, 1593 cm^{-1}). The mass spectrum did not provide a molecular ion.

The ^{1}H n.m.r. spectrum shows signals for two methyl groups (δ 1·49 and 1·71), two hydroxy groups (δ 3·49 and 10·0), a sorboyl group, and an isolated three-spin system (δ 2·77 (dd, J 16, 10 Hz, 1H), 3·15 (dd, *J* 16, 2 Hz, 1H) and 4·14 (dd, *J* 10, 2 Hz, 1H)). The ^{13}C n.m.r. spectrum shows 14 signals corresponding to methyl carbons (δ 5·3 and 18·1), a methylene carbon (δ 40·5), a secondary carbon bearing oxygen (δ 70·3), a tertiary carbon bearing oxygen (δ 82·8), four sp^2 carbons as doublets, and three carbonyl-like signals (δ 173·4, 175·4 and 198·5). The information from the ^{1}H and ^{13}C n.m.r. spectra leads to the molecular formula $C_{14}H_{18}O_5$ for compound (1). The appearance of a methyl signal at δ 5·3 together with the signals at δ 173·4, 175·4, 82·8 and 96·4 in the ^{13}C n.m.r. spectrum as singlets strongly suggests the presence of a tetronic acid moiety.[3] The secondary alcohol (δ 4·14 and δ 70·3 in ^{1}H and ^{13}C n.m.r., respectively) hydrogen is part of a three-spin system which, together with the evidence for a CH_2 group, allows us to derive the fragment $CH(OH)CH_2CO$. The large coupling constant (16 Hz) observed in the ^{1}H n.m.r. spectrum between the hydrogens at δ 3·15 and 3·49 indicates that these hydrogens are geminally coupled and α to a carbonyl group. The carbonyl group in the above fragment may be assigned to the sorboyl carbonyl, since the remaining three oxygen atoms belong to the tetronic acid moiety. Thus, the constitution of this compound is assigned as 5-hydroxyvertinolide (1).

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† Dedicated, with respect, to Professor R. C. Cambie.

The possible biosynthetic relationship between 5-hydroxyvertinolide and the previously described vertinolide,[3] sorbiquinol,[2] and bisvertinols,[1,4] which possess the (*S*)-configuration at the stereogenic centre corresponding to C 4 of 5-hydroxyvertinolide, leads us to assign the (*S*)-configuration at C 4 in this compound. The absolute configuration at C 5 was not determined, due to the small quantities available. Attempts to reisolate this compound were unsuccessful. The parent compound vertinolide was isolated from later cultures rather than 5-hydroxyvertinolide.

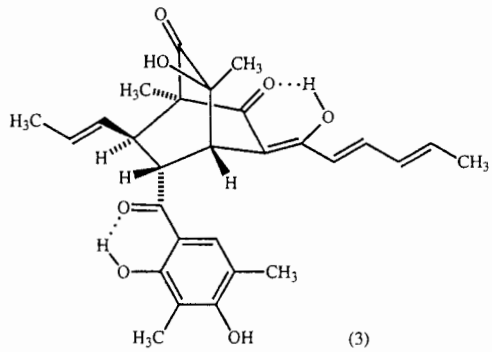
Bislongiquinolide was obtained as an yellow gum, $[\alpha]_D$ +105° (MeOH). The maxima in the u.v. spectrum (262, 291, 370 and 385sh nm) shift to longer wavelenghts upon addition of base indicating the acidic nature of the chromophoric group. Its i.r. spectrum shows absorptions for hydroxy groups (3400 cm^{-1}), ketone carbonyl (1732 cm^{-1}), strongly chelated carbonyl (1660 and 1620 cm^{-1}), and double bonds (1602 cm^{-1}).

The molecular formula of bislongiquinolide, $C_{28}H_{32}O_8$, is obtained from the high-resolution e.i. mass spectrum and confirmed by c.i. mass spectrometry. The mass spectrum shows fragment ions corresponding to loss of water (*m/z* 478), loss of CO (*m/z* 468), loss of a sorboyl chain (*m/z* 401), the consecutive loss of CO and a sorboyl chain (*m/z* 373), and an ion at *m/z* 95 (C_6H_7O) corresponding to the sorboyl group.

The ^{1}H n.m.r. spectrum displays four methyl singlets (δ 1·10, 1·28, 1·49 and 1·56), signals of two sorboyl chains, an enolized β-dicarbonyl system (δ 14·0), a three-hydrogen spin system (δ 3·18 (dd, *J* 6, 2 Hz, 1H), 3·34 (d, *J* 2 Hz, 1H) and 3·39 (d, *J* 6 Hz, 1H)), and three hydroxy hydrogens (δ 5·0, 10·0 and 14·0). The stereochemistry of the sorboyl chains is assigned as *E*,*E* on the basis of the coupling constants (15 Hz) of the signals at δ 6·12, 6·16, 7·23 and 7·34.

The ^{13}C n.m.r. spectrum of bislongiquinolide shows six methyl carbons (δ 6·3, 11·1, 19·0, 19·2, 23·2 and 23·5), three methine carbons (δ 42·4, 43·6 and 51·3), a quaternary carbon (δ 62·6), two tertiary carbons bearing oxygens (δ 74·7 and 82·9), two electron-rich sp^2 carbons (δ 98·2 and 108·5), eight sp^2 carbons as doublets, and six carbonyl-like carbons (δ 169·7, 174·2, 176·1, 194·9, 202·6 and 208·2). The low-field signal (δ 82·9) for the tertiary carbon bearing oxygen suggests that the oxygen is acylated. This, together with the diagnostic high-field methyl signal (δ 6·3), and the singlets at δ 98·2, 174·2 and 176·1, is strong evidence for the presence of a tetronic acid moiety (see below). This part structure accounts for the hydroxy hydrogen at δ 10·0 and the methyl singlets at δ 1·56 and 1·49 in the ^{1}H n.m.r. spectrum of bislongiquinolide (see Experimental). The presence of the tetronic acid moiety in bislongiquinolide was confirmed by a series of selective INEPT experiments. Selective irradiation of the methyl hydrogens at δ 1·56 (CH_3–C 23) gave signals at δ 98·2 (C 23), 174·2 and 176·1 (C 22 and C 24), while irradiation of the methyl hydrogens at δ 1·49 (CH_3–C 21) gave signals at δ 82·9 (C 21), 98·2 (C 23), 174·2 and 176·1 (C 22 and C 24).

The signals at δ 169·7 and 194·9 may be assigned to the enolized β-dicarbonyl system, and the signals at δ 202·6 and 208·2 to the a sorboyl carbonyl and a ketone carbonyl, respectively, on the basis of chemical shifts. The presence of an alcohol hydroxy hydrogen in the ^{1}H n.m.r. spectrum (δ 5·0) is consistent with the presence of a tertiary carbon (δ 74·7) in the ^{13}C n.m.r. spectrum. Since all the carbonyl signals are assigned, the sorboyl carbonyl group must be part of the enolized β-dicarbonyl system.



(3)

The n.m.r. signals of the sorboyl–enol system, together with the presence of an unconjugated ketone and a tertiary alcohol, are very similar to those of the dimeric metabolite sorbiquinol (3).[2] This combined with the evidence for the presence of a tetronic acid fragment and of a second sorboyl group allows us to deduce structure (2) for bislongiquinolide. This structure is further supported by INEPT experiments. Thus, irradiation of the methyl hydrogens at δ 1·10 (CH_3–C 1) gave signals in the ^{13}C n.m.r. spectrum for the quaternary carbon (C 1, δ 62·6), the ketone carbonyl (C 6, δ 208·2), and the chelated enol system carbon (C 2, δ 194·9), while irradiation of the methyl hydrogen at δ 1·28 (CH_3–C 5) gave signals for the tertiary carbon (C 5, δ 74·7) and the ketone carbonyl (C 6, δ 208·2). The high-field shift observed for C 2 indicates that it is ketonic in nature and thus dictates that the direction of enolization of the β-dicarbonyl system is as depicted in (2). This assignment is in agreement with the long-wavelength absorptions (370 and 385 nm) observed in the u.v. spectrum and also with the type of enolization observed in the structurally related metabolites bisvertinolone[5] and bisvertinoquinol.[6] We now believe that sorbiquinol[2] also is enolized in this way, as depicted in (3).

A series of n.O.e. experiments in $CDCl_3/C_6D_6$ shows enhancement of the methine hydrogen at δ 3·39 (H 4) upon irradiation of the methyl hydrogens at δ 1·28 (CH_3–C 5), while irradiation at δ 3·39 (H 4) gave enhancement of the sorboyl chain hydrogen at δ 6·1 (H 10) and at 7·32 (H 11), and of the methine hydrogen at δ 3·26 (H 8). Irradiation of the methine hydrogen at δ 3·49 (H 7) gave enhancement of the methine hydrogen

at δ 3·26 (H 8) and the sorboyl chain hydrogens at δ 6·1 (H 16) and 7·18 (H 17), while irradiation of H 8 (δ 3·26) gave enhancement of H 4 and H 7. In addition, irradiation of the methyl group hydrogens at δ 1·15 (CH_3–C 1) gave enhancement of the sorboyl chain hydrogen at δ 6·1 (H 16) and of the methine hydrogen at δ 3·49 (H 7). Irradiation of the methyl group at δ 1·48 (CH_3–C 21) gave enhancement of the methine hydrogens at δ 3·26 (H 8), 3·39 (H 4) and 3·49 (H 7).

The n.O.e. experiments in $CDCl_3$ gave the following results. Irradiation of the methyl hydrogens at δ 1·28 (CH_3–C 5) gave enhancement at δ 3·34 (H 4), while simultaneous irradiation of H 4 (δ 3·34) and H 7 (δ 3·39) gave enhancement of the signals at δ 6·12 and 6·16 (H 10 and H 16) of the two sorboyl chains. The methyl at δ 1·49 (CH_3–C 21) gave enhancements at δ 3·18 (H 8), 3·34 (H 4) and 3·39 (H 7).

The n.O.e. results support structure (2) for bislongiquinolide, but do not solve the relative stereochemistry at C 7 and C 8.

The similarity between the coupling constants $J_{H4/H8}$ (2 Hz) and $J_{H7/H8}$ (6 Hz) of bislongiquinolide and those of sorbiquinol (1·5 and 6·5 Hz, respectively), whose stereochemistry is known,[2] allows us to infer the *trans* relationship shown in structure (2).

The relative stereochemistry at C 5 is based on the chemical shift of the methyl group (CH_3–C 5, δ 1·28) which is at high field for a methyl group at a carbon bearing oxygen, and can be attributed to the shielding effect of the adjacent enolized β-dicarbonyl system.

Experimental

For general experimental details and isolation of compounds (1) and (2) see ref. 2.

5-Hydroxyvertinolide (1). Colourless amorphous solid, $[\alpha]_D$ −64° (*c*, 0·11 in MeOH). I.r. ν_{max} 3360, 3025, 2987, 2965, 2936, 1749, 1729, 1666, 1638, 1593, 1447, 1402, 1306, 1064, 1020, 762 cm^{-1}. U.v. λ_{max} (ϵ) in MeOH: 230sh, 263 (13000), 359 nm (900); in NaOH: 203 (19000), 260 (16000), 373 nm (700); in HCl: 229 (8100), 274 (10400), 357 nm (1100). ^{1}H n.m.r. δ (360 MHz, (D_6)acetone) 1·49, s, 3H, CH_3–C 4; 1·74, s, 3H, CH_3–C 2; 1·90, d, *J* 6·5 Hz, 3H, H 12; 2·77, dd, *J* 16, 10 Hz, 1H, H 6; 3·15, dd, *J* 16, 2 Hz, 1H, H 6; 3·49, s, 1H, C 5–OH; 4·14, dd, *J* 10, 2 Hz, 1H, H 5; 6·08, d, *J* 15 Hz, 1H, H 8; 6·1–6·4, m, 2H, H 10, H 11; 7·21, dd, *J* 16, 10 Hz, 1H, H 9; 10·0, br s, 1H, C 3–OH. ^{13}C n.m.r. δ (100 MHz, (D_6)acetone) 5·3, C 13; 18·1, C 12, C 14; 40·5, C 6; 70·3, C 5; 82·8, C 4; 96·4, C 2; 127·2, C 8; 129·7, C 10; 140·6, C 11; 143·5, C 9; 173·4, C 1; 175·4, C 3; 198·5, C 7.

Bislongiquinolide (2). Yellow gum, $[\alpha]_D$ +105° (*c*, 0·55 in MeOH). I.r. ν_{max} 3400, 3050, 2980, 2920, 1732, 1660, 1626, 1602, 1563, 1380, 1065, 998 cm^{-1}. U.v. λ_{max} (ϵ) in MeOH: 262 (19000), 292 (20000), 370 (15000), 385sh nm; in NaOH: 204 (83000), 259 (21000), 287 (22000), 393 nm (11000); in HCl: 232 (11000), 294 (18000), 368 (17000), 385sh nm. C.d. (MeOH) λ_{extr} ($\Delta\epsilon$): 360 (+11), 320 (−18), 281 (+7), 230 nm (−8). O.r.d. (MeOH) λ_{extr} ([ϕ]): 390 (+26000), 338 (−64000), 300 (+67000), 267 nm (+4500). ^{1}H n.m.r. δ (360 MHz, $CDCl_3$) 1·10, s, 3H, CH_3–C 1; 1·28, s, 3H, CH_3–C 5; 1·49, s, 3H, CH_3–C 21; 1·56, s, 3H, CH_3–C 23; 1·90, d, *J* 7·0 Hz, 1H, H 14 or H 20; 1·92, d, *J* 7·0 Hz, 1H, H 20 or H 14; 3·18, dd, *J* 6, 2 Hz, 1H, H 8; 3·34, d, *J* 2 Hz, 1H, H 4; 3·39, d, *J* 6·0 Hz, 1H, H 7; 5·0, br s, 1H, C 5–OH; 6·12, d, *J* 15·0 Hz, 1H, H 10 or H 16; 6·16, d, *J* 15·0 Hz, 1H, H 16 or H 10; 6·20–6·45, m, 5H; 7·23, dd, *J* 15·0, 10·5 Hz, 1H, H 17; 7·34, dd, *J* 15·0, 10·5 Hz, 1H, H 11; 10·0, br s, 1H, C 22–OH; 14·02, br s, 1H, C 2–OH. N.O.e. ($CDCl_3/C_6D_6$) [1·15] (CH_3–C 1): 6·1 (H 16, −3·6%), 3·49 (H 7, −1%); [1·28] (CH_3–C 5): 3·39 (H 4, −2%); [3·26] (H 8): 3·39 (H 4, −8·5%), 3·49 (H 7, −11%); [3·39] (H 4): 6·1 (H 10, −14·4%), 3·26 (H 8, −10%), 7·32 (H 11, −2%); [3·49] (H 7): 3·26 (H 8, −5%), 6·1 (H 16, −8%), 7·18 (H 17, −6%). ^{13}C n.m.r. δ (100 MHz, $CDCl_3$) 6·3, **C**H_3–C 23; 11·1, **C**H_3–C 5; 19·0, 19·2, C 14, C 20; 23·2, 23·5, **C**H_3–C 1, **C**H_3–C 21; 42·4, 43·6 and 51·3, C 4, C 7 and C 8; 62·6, C 1; 74·7, C 5; 82·9, C 21; 98·2, C 23; 108·5, C 3; 117·6, 127·0, 130·2, 130·9, 140·6, 143·8, 145·4, 147·7, sorboyl CH; 169·7, C 9; 174·2, C 24; 176·1, C 22; 194·9, C 2; 202·6, C 15; 208·2, C 6. INEPT: [1·10] (C**H**$_3$–C 1): 62·6, C 1, 194·9, C 2, 208·2, C 6; [1·28] (C**H**$_3$–C 5): 74·7, C 5, 208·2, C 6. E.i. mass spectrum *m/z* 496 (52%), 453 (22), 373 (55), 248 (100), 232 (70), 217 (30), 180 (62), 95 (93). High-resolution e.i. mass spectrum *m/z* 496·2089 ($C_{28}H_{32}O_8$ requires 496·2098).

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