Structures of Virescenosides D and H, New Metabolites of *Acremonium luzulae* (Fuckel) Gams †

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Two new metabolites, virescenosides D (3a) and H (4a), isolated from the fungus *Acremonium luzulae* (Fuckel) Gams, are shown to be the 4-deoxy-β-D-*threo*-hex-4-enodialdopyranoside and the 4-deoxy-β-D-*threo*-hex-4-enopyranoside of virescenol B (5b), respectively, on the basis of chemical and spectral evidence and, particularly, their partial synthesis from the known virescenoside B (1b).

OUR chemical investigation on the constituents of the fungus *Acremonium luzulae* (Fuckel) Gams † resulted in the isolation of five metabolites: virescenosides A (1a) and B (1b),[1] C (1c),[2] and F (2a) and G (2b).[3] These were the first natural altrose and altruronic acid glycosides to be reported, each derived from one of three isopimaradienic aglycones, called virescenols A (5a),[4]

† This fungus has been classified lately as *Oospora virescens* (Link) Wallr.

[1] N. Cagnoli-Bellavita, P. Ceccherelli, M. Ribaldi, J. Polonsky, and Z. Baskevitch, *Gazzetta*, 1969, 1354.

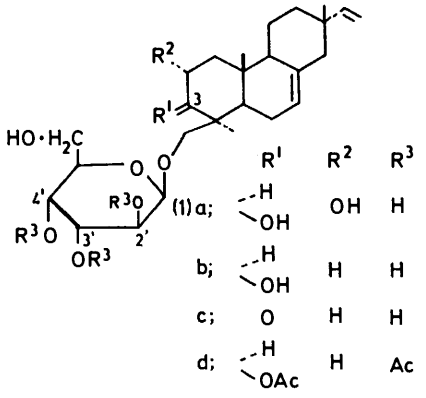
[2] N. Cagnoli-Bellavita, P. Ceccherelli, R. Mariani, J. Polonsky, and Z. Baskevitch, *European J. Biochem.*, 1970, **15**, 356.

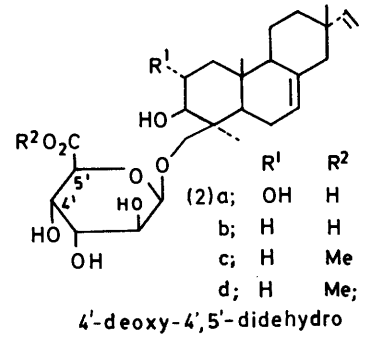
[3] P. Ceccherelli, N. Cagnoli-Bellavita, J. Polonsky, and Z. Baskevitch, *Tetrahedron*, 1973, **29**, 449.

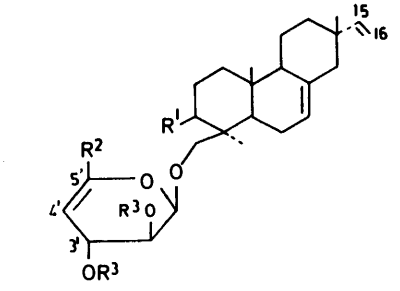
[4] J. Polonsky, Z. Baskevitch, N. Cagnoli-Bellavita, and P. Ceccherelli, *Chem. Comm.*, 1968, 1404; *Bull. Soc. chim. France*, 1970, 1912.

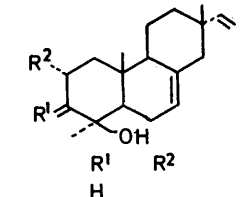
B (5b),[4] and C (5c).[2] We now describe the isolation of two new glycosides, virescenosides D and H,* for which we propose structures (3a) and (4a), respectively.

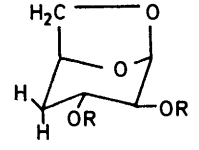
Acidic hydrolysis of both virescenosides D (3a) and H (4a) led to the same aglycone, virescenol B (5b); thus, the two new virescenosides were seen to differ in virescenoside H (4a), which has no carbonyl group and which possesses an additional hydroxy-group with respect to (3a). That this is the only difference between the two metabolites was shown by the ready conversion of virescenoside D (3a) into virescenoside H (4a) by reduction with borohydride.

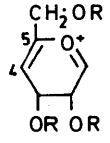












the nature of the sugar unit. Acetylation yielded a triacetate (3b) and a tetra-acetate (4b), respectively.

The spectral data of (3a) [δ 9.23 (1 H, s) and 5.94 (1 H, d); $\lambda_{max.}$ 258 nm; $\nu_{max.}$ 1 690 cm^{-1}] indicated the presence, in the carbohydrate portion, of an αβ-unsaturated aldehyde group. This is replaced by an αβ-unsaturated primary alcohol group in the more polar

Catalytic hydrogenation of virescenoside H (4a), led to the non-crystalline tetrahydrovirescenoside H (4c), giving a tetra-acetate (4d). The sugar component of the saturated compound (4c) was isolated, by mild acidic hydrolysis, as the crystalline 1,6-anhydro-4-deoxy-β-D-*arabino*-hexopyranose (6a),[5] which on acetylation gave the diacetate (6b).† The same configuration

* A preliminary communication on the structure of virescenoside H was presented at the 7th International Symposium on the Chemistry of Natural Products, Riga, 1970, Abstracts, p. 399.

† The values of the specific rotations of compounds (6a and b) are in good agreement with those calculated by Horton's method.[6]

[5] (*a*) M. Černý and J. Pacák, *Coll. Czech. Chem. Comm.*, 1962, **27**, 94; (*b*) A. F. Cook and W. G. Overend, *J. Chem. Soc.* (*C*), 1966, 1549.

[6] D. Horton and J. D. Wander, *Carbohydrate Res.*, 1970, **14**, 83.

at C-2′ and C-3′ is found in the known virescenosides; 1,6-anhydro-D-altropyranose (altrosan) was isolated from acidic hydrolysis of virescenosides A, B,[1] and C.[2]

Structures (3a) and (4a) for virescenosides D and H were confirmed by their correlation, in two different ways, with virescenoside B (1b) as follows. (i) The methyl ester (2c) of virescenoside G, which has been previously [3] correlated with virescenoside B (1b), was treated with sodium methoxide according to the procedure of Heim and Neukom.[7] Reduction of the resulting αβ-unsaturated ester (2d) with lithium aluminium hydride led to virescenoside H (4a). (ii) Tritylation of virescenoside B (1b), followed by acetylation and detritylation, afforded the tetra-acetate (1d) of virescenoside B. When submitted to an oxidative elimination according to the procedure of Cree *et al.*[8] (by using Parikh and Doering's oxidant [9]), the tetra-acetate (1d) gave, in good yield, the triacetate (3b) of virescenoside D. The compound obtained by mild deacetylation was identical with virescenoside D (3a).

The foregoing results prove that virescenosides D (3a) and H (4a) are the 4-deoxy-β-D-*threo*-hex-4-enodialdopyranoside and the 4-deoxy-β-D-*threo*-hex-4-enopyranoside of virescenol B (5b), respectively. Virescenoside D is, to our knowledge, the first reported naturally occurring glycoside to have an αβ-unsaturated aldehyde group in its sugar unit.

Virescenoside D proved to be an effective antimycotic agent.[10] Virescenosides A and B, which are the major glycosides of the fungus in question, do not display this activity. The above described reactions [(1b) ⟶ (1d) ⟶ (3a)] enable the preparation of the antifungitoxic virescenoside D from the inactive virescenoside B.

Two other metabolites, virescenosides E and L have recently been isolated; [11] they differ from (3a) and (4a) in the nature of the aglycone (virescenol A instead of virescenol B).

EXPERIMENTAL

M.p.s were taken with a Kofler hot-stage apparatus, i.r. spectra with a Perkin-Elmer 257 grating spectrometer, and u.v. spectra with a Bausch and Lomb spectrophotometer. Mass spectra were recorded with an A.E.I. MS9 instrument and n.m.r. spectra (60 MHz) with a Varian A-60 spectrometer ($CDCl_3$ as solvent and Me_4Si as internal standard). Optical rotations were measured on a Roussel-Jouan Quick polarimeter.

Isolation of Virescenosides D (3a) *and H* (4a).—Lyophilised culture (15 g) of *Acremonium luzulae* (Fuckel) Gams was extracted with ethanol (Soxhlet). The extract was concentrated to dryness and the residue (6 g) was chromatographed on a column of silica gel. Elution with chloroform–methanol (93 : 7) afforded the metabolites (3a) (220 mg) and (4a) (80 mg); t.l.c. ($CHCl_3$–MeOH, 85 : 15) R_F 0.7 and 0.5, respectively. *Virescenoside D* (3a) gave a white precipitate from chloroform–hexane, $[\alpha]_D$ −123.5° (*c* 0.96 in MeOH) (Found: C, 70.15; H, 8.5. $C_{26}H_{38}O_6$ requires C, 69.95 : H, 8.6%); *m/e* 446 (M^+) and 428 (M^+ − 18); δ 0.79, 0.85, and 1.14 (each 3 H, s, Me), 3.37 (2 H, m, H-3 and -2′), 3.75 and 4.12 [each 1H, dd, *J* 8.5 Hz, CH_2·O (AB system)], 5.33 (1 H, m, H-7), 5.94 (1 H, d, *J* 3 Hz, H-4′), and 9.23 (1 H, s, CHO). *Virescenoside H* (4a). afforded needles (from ethyl acetate), m.p. 190—193°, $[\alpha]_D$ −149° (*c* 0.72 in MeOH) (Found: C, 69.85; H, 9.2. $C_{26}H_{40}O_6$ requires C, 69.6; H, 9.0%); *m/e* 448 (M^+), 430 (M^+ − 18), 346, 287, 145 [ion (7)], 109 [ion (7) − 36]; δ 0.81, 0.85, and 1.14 (each 3 H, s, Me), 3.38 (2 H, m, H-3 and -2′), 3.7 and 4.25 (each 1 H, d, *J* 8.5 Hz, CH_2·O), 3.95 (2 H, s, CH_2·OH), and 5.33 (1 H, m, H-7).

Acidic Hydrolysis of the Virescenosides (3a) *and* (4a).—A solution of virescenoside D (3a) (150 mg) in 0.5M-hydrochloric acid–tetrahydrofuran (5 ml) was heated on a boiling water-bath for 2 h, cooled, diluted with water, and extracted with chloroform to give a solid (90 mg). Recrystallisation from ether–petroleum yielded virescenol B as needles, m.p. 146—147°, $[\alpha]_D$ −25° (*c* 0.71 in $CHCl_3$). Hydrolysis of virescenoside H (4a) under the same conditions also afforded virescenol B.

Acetylation of the Virescenosides (3a) *and* (4a).—Acetic anhydride (3 ml) was added to a solution of virescenoside D (3a) (220 mg) in pyridine (3 ml). The solution was left for 24 h at ambient temperature, then treated with ice–water. Extraction of the acidified solution with chloroform afforded a solid which was purified by chromatography on silica gel. Benzene–ethyl acetate eluted a solid (200 mg) which on recrystallisation from ether–hexane or 95% ethanol afforded the *triacetate* (3b), m.p. 123—124°, $[\alpha]_D$ −183° (*c* 1.02 in $CHCl_3$) (Found: C, 67.35; H, 7.4. $C_{32}H_{44}O_9$ requires C, 67.1; H, 7.75%); *m/e* 572 (M^+), 512, and 452; δ 0.85, 0.88, and 0.96 (each 3 H, s, Me), 2.05, 2.1, and 2.12 (each 3 H, s, Ac), 3.63 and 4.26 (each 1 H, d, *J* 10 Hz, CH_2·O), 5.36 (1 H, m, H-7), 5.90 (1 H, d, *J* 3 Hz, H-4′), and 9.23 (1 H, s, CHO). Acetylation of virescenoside H (4a) (40 mg) under the same conditions afforded a solid which was purified by chromatography on silicic acid to give the *tetra-acetate* (4b) as a non-crystalline solid (Found: C, 65.9; H, 7.55. $C_{34}H_{48}O_{10}$ requires C, 66.2; H, 7.85%); *m/e* 616 (M^+), 556, and 496; δ 0.87, 0.9, and 0.98 (each 3 H, s, Me), 2.1, 2.12, and 2.13 (each s, 6 H, 3 H, and 3 H, Ac), 3.63 and 4.28 (each 1 H, d, *J* 10 Hz, CH_2·O), 4.55 (2 H, s, CH_2·OAc), and 5.42 (1 H, m, H-7).

Reduction of Virescenoside D (3a).—Sodium borohydride (40 mg) was added to a solution of virescenoside D (3a) (54 mg) in methanol (2 ml) at 22 °C. After 15 min the solution was diluted with water. Extraction of the acidified solution with chloroform yielded material which was chromatographed on silicic acid. Chloroform containing methanol (5%) eluted a solid (30 mg), which on recrystallisation from ethyl acetate afforded virescenoside H (4a) as needles, m.p. 190—193°.

Hydrogenation of Virescenoside H (4a).—Virescenoside H (4a) (41 mg) in ethanol containing Adams catalyst (25 mg) was hydrogenated for 1 h at room temperature and atmospheric pressure. The product was separated by chromatography on silicic acid (4 g); chloroform–methanol (97 : 3) eluted tetrahydrovirescenoside H (4c), obtained as a non-

[7] P. Heim and H. Neukom, *Helv. Chim. Acta*, 1962, **45**, 1735.
[8] G. M. Cree, D. W. Mackie, and A. S. Perlin, *Canad. J. Chem.*, 1969, **47**, 511; N. Bourguignon-Zylber and J. Polonsky, *Biochimie*, 1971, **53**, 263.
[9] J. R. Parikh and W. Von E. Doering, *J. Amer. Chem. Soc.*, 1967, **89**, 5505.
[10] N. Cagnoli-Bellavita, U.S.P. 3801 565 (*Chem. Abs.*, 1974, 103 227 f).
[11] N. Cagnoli-Bellavita, P. Ceccherelli, M. Ribaldi, J. Polonsky, and Z. Baskevitch-Varon, *Gazzetta*, in the press.

crystalline solid, $[\alpha]_D$ −56.1° (*c* 1.01 in MeOH); *m/e* 452 (M^+) and 434 ($M^+ - 18$); δ 0.68, 0.8, and 1.18 (each 3 H, s, Me), 3.93 (2 H, s, CH_2·OH), and 5.33 (1 H, m, H-7). Acetylation of the product (4c) with acetic anhydride–pyridine affords the *tetra-acetate* (4d) as a non-crystalline solid (Found: M^+,620.357 9. $C_{34}H_{52}O_{10}$ requires *M*, 620.355 9); *m/e* 560, 500, and 273 [ion (8)]; δ 0.72, 0.9, and 0.97 (each 3 H, s, Me), 2.05, 2.08, and 2.13 (each s, 3 H, 3 H, and 6 H, Ac), 3.58 and 4.33 (each 1 H, d, *J* 10 Hz, CH_2·O), and 5.33 (1 H, m, H-7).

Acidic Hydrolysis of Tetrahydrovirescenoside H (4c).—Compound (4c) (300 mg) was hydrolysed in the same way as (3a). Extraction with chloroform afforded 15,16-dihydrovirescenol B (189 mg). The concentrated aqueous solution was neutralised with Bio-RAD resin [AG × 8 (D) 50; 7 ml] to give a syrup (74 mg). Recrystallisation from ether–hexane yielded, 1,6-anhydro-4-deoxy-β-D-*arabino*-hexopyranose (6a) as prisms, m.p. 105—106°, $[\alpha]_D$ −169° (*c* 0.8 in MeOH) (lit.,[5a] m.p. 105—107°, $[\alpha]_D$ −164°); *m/e* 129 ($M^+ - 17$), 128 ($M^+ - 18$), 115 ($M^+ - CH_2OH$); δ (D_2O) 5.41 (1 H, d, *J* 3 Hz, H-1) and *ca.* 2.05 (2 H, m, H-4).

Acetylation of the Sugar (6a).—Acetic anhydride (0.5 ml) was added to a solution of compound (6a) (38 mg) in pyridine (0.5 ml). After 24 h at room temperature the usual work-up afforded an oil (42 mg), which was chromatographed on silicic acid (2 g) to give 2,3-*di*-O-*acetyl*-1,6-*anhydro*-4-*deoxy*-β-D-*arabinose* (6b) (31 mg) as a syrup, b.p. 120° (bath temp.) at 0.02 mmHg, $[\alpha]_D$ −123.8° (*c* 1.01 in MeOH) (Found: C, 52.1; H, 6.1. $C_{10}H_{14}O_6$ requires C, 52.15; H, 6.15%); *m/e* 170 ($M^+ - 60$) and 128 ($M^+ - 60 - 42$); δ 2.02 and 2.1 (each 3 H, s, Ac), *ca.* 2.15 (2 H, m, H-4), and 5.43 (1 H d, *J* 3 Hz, H-1).

Virescenoside H (4a) *from Virescenoside G Methyl Ester* (2c).—A solution of sodium (75 mg) in dry methanol (5 ml) was added to a solution of the methyl ester (2c)[3] (500 mg) in dry methanol (10 ml). The solution was heated under reflux for 30 min, cooled, neutralised with formic acid, and evaporated to dryness. A chloroform extract of the residue gave a residue which was chromatographed on a silica gel column. Chloroform–methanol (92 : 8) eluted the αβ-unsaturated ester (2d) (130 mg), homogeneous by t.l.c. which was further reduced without purification. To a solution of this substance in dry tetrahydrofuran (8 ml) was added an excess of lithium aluminium hydride. After 3 h at 50 °C the excess of hydride was destroyed with ethyl acetate and acetic acid. Extraction with chloroform gave a product (122 mg) which was chromatographed on a silica gel column. Recrystallisation from ethyl acetate gave pure virescenoside H (4a).

2′,3,3′,4′-*Tetra-O-acetylvirescenoside B* (1d).—Triphenylmethyl chloride (1.5 g) was added to a solution of virescenoside B (1b) (2 g) in dry pyridine (10 ml). After 4 days, at 20 °C acetic anhydride (14 ml) was added and the mixture was stored at room temperature for another 3 days. It was then poured on crushed ice, and extracted with chloroform. The extract was washed with water, dried, and evaporated and a solution of the residue (3.8 g) in glacial acetic acid (70 ml) was treated at room temperature with a solution of hydrogen bromide in glacial acetic acid (1%; 150 ml). After 2 h the mixture was poured into water and extracted with chloroform. The extract was washed with dilute aqueous sodium hydrogen carbonate and water, dried, and evaporated, and the residue (3.7 g) was chromatographed on a silicic acid–Celite (5 : 1) column [benzene–ether (8 : 2) as eluant]. Fractions were monitored by t.l.c. [benzene–ether (1 : 1)]. The first substance eluted was triphenylmethanol, the second was the known penta-acetate of virescenoside B[1] (200 mg), m.p. 143—145°, $[\alpha]_D$ −39.1° (*c* 1.1 in $CHCl_3$), and the third was the *tetra-acetate* (1d) of virescenoside B (1.6 g), which on recrystallisation from ether afforded prisms, m.p. 153—155°, $[\alpha]_D$ −35.4° (*c* 1.27 in $CHCl_3$) (Found: C, 64.2; H, 7.9. $C_{34}H_{50}O_{11}$ requires C, 64.35; H, 7.9%); *m/e* 634 (M^+), 616 ($M^+ - 18$), and 603 ($M^+ - 31$); δ 0.86, 0.92, and 0.96 (each 3 H, s, Me), 2.03, 2.06, and 2.15 (each s, 3 H, 3 H, and 6 H, Ac), and 3.61 and 4.3 (each 1 H, d, *J* 10 Hz, CH_2·O).

Oxidative Elimination Reaction of the Tetra-acetate (1d).—A solution of pyridine–sulphur trioxide complex (0.41 g) in dimethyl sulphoxide (3 ml) was slowly added to a mixture of the tetra-acetate (1d) (0.31 g), dimethyl sulphoxide (1.4 ml), and triethylamine (1 ml). After 40 min at room temperature, the mixture was diluted with chloroform, and washed successively with cold saturated tartaric acid and water. Removal of the solvent left a residue (0.32 g) which was chromatographed on a column of silicic acid–Celite (2 : 1). Elution with benzene–ether (9 : 1) gave a solid (0.185 g), which on recrystallisation (ethanol) afforded needles, m.p. 123—124°, identical, with 2′,3,3′-tri-*O*-acetylvirescenoside D (3b).

Deacetylation of the Triacetate (3b).—The triacetate (3b) (0.28 g) was deacetylated in methanol (19 ml) with sodium (0.14 g) at room temperature during 1.5 h. The solution was neutralised by filtering through a column of Amberlite IR-120 resin. The residue (0.255 g) obtained after evaporation of the eluate was chromatographed on a column of silicic acid–Celite (3 : 1). The substance (0.2 g) eluted with chloroform–methanol (9 : 1) was identical with natural virescenoside (3a) (R_F on t.l.c., $[\alpha]_D$, and mass and n.m.r. spectra).

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