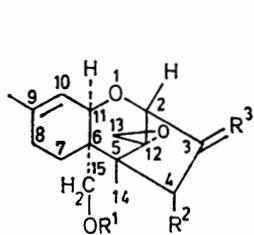


Calonectrin and 15-Deacetylcalonectrin, New Trichothecanes from *Calonectria nivalis*

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Two new metabolites, calonectrin and 15-deacetylcalonectrin, isolated from culture filtrates of *Calonectria nivalis*, have been shown to be 3 α ,15-diacetoxy-12,13-epoxytrichothec-9-ene (1) and 3 α -acetoxy-12,13-epoxytrichothec-9-en-15-ol (2), respectively. The known sesquiterpene culmorin (13) has also been isolated.

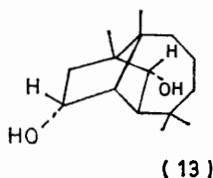
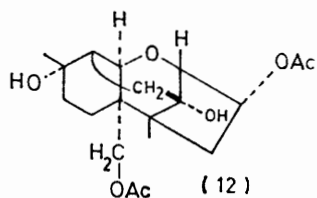
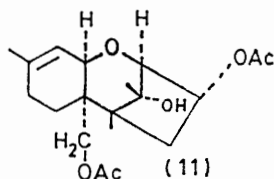
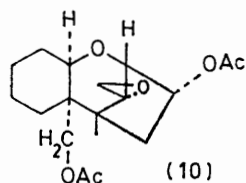
THE most numerous class of fungal sesquiterpenes is that based on the trichothecane skeleton.¹ We have isolated from *Calonectria nivalis* two new members of the class, for which we propose the names calonectrin and 15-deacetylcalonectrin and the structures (1) and (2),



	R ¹	R ²	R ³
(1)	Ac	H	α -OAc, β -H
(2)	H	H	α -OAc, β -H
(3)	H	H	α -OH, β -H
(4)	Ac	H	α -OH, β -H
(5)	Ac	H	O
(6)	Ac	OAc	α -OH, β -H
(7)	H	H	O
(8)	Ac	H	α -O ₂ CEt, β -H
(9)	Ac	H	α -O ₂ CPr, β -H

oxy-substituent at C-4. *C. nivalis* also produces the known sesquiterpene culmorin (13), previously isolated from *Fusarium culmorum*.³

The n.m.r. spectra (Table) of calonectrin and deacetylcalonectrin are similar, the significant differences being that the former has two acetyl signals and an AB quartet (*J* 12 Hz) centred at τ 6.03, whereas the latter has one acetyl signal, one exchangeable proton, and an AB quartet centred at τ 6.44. These features suggest that a primary acetate system in (1) is replaced by a free hydroxy-group in (2); the i.r. spectra of the compounds are consistent with this difference. Both metabolites can be hydrolysed to the same diol (3), suggesting that the only difference between them is the extent of acetylation. The n.m.r. spectra also show signals due to a tertiary methyl group, and the following part structures, the presence of which was supported by the results of spin-decoupling experiments: CH₃·C=CH·CH;



$>C(O)-CH_2$; and CH-CH(OAc)-CH₂. These features are consistent with calonectrin being 3 α ,15-diacetoxy-12,13-epoxytrichothec-9-ene (1) or the 14-acetoxy-isomer.

Chemical proof of the structure of calonectrin was provided by the following experiments. Partial hydrolysis to the 3-hydroxy-derivative (4) followed by oxidation with silver carbonate-Celite reagent⁴ gave the 3-ketone (5) with properties in agreement with those reported⁵ for the compound obtained from diacetoxyscirpenol (6). Further hydrolysis of the ketone (5) yielded the 15-hydroxy-ketone (7), identical with a sample prepared from diacetoxyscirpenol by partial hydrolysis, methylsulphonylation, and subsequent treat-

respectively. Unlike the previously described members of the class,² the *C. nivalis* metabolites do not possess an

¹ W. O. Godtfredsen, J. F. Grove, and Ch. Tamm, *Helv. Chim. Acta*, 1967, **50**, 1666.

² W. B. Turner, 'Fungal Metabolites,' Academic Press, London, 1971, p. 219.

³ J. N. Ashley, B. C. Hobbs, and H. Raistrick, *Biochem. J.* 1937, **31**, 385; D. H. R. Barton and N. H. Werstiuk, *J. Chem. Soc. (C)*, 1968, 148.

⁴ V. Balogh, M. Fetizon, and M. Golfier, *Angew. Chem. Internat. Edn.*, 1969, **8**, 444.

ment with sodium ethoxide.⁵ The stereochemistry at C-3 follows from the coupling constant (5 Hz) between H-2 and H-3, which is in agreement with that observed in other 3-substituted trichothecanes⁶ and indicates that the 3-acetoxy-group of calonecetrin has the α -configuration.

We have prepared several other derivatives of calonecetrin. Hydrogenation of calonecetrin gave the dihydro-derivative (10) which, from the sharpness of its n.m.r. spectrum and its m.p., appears to be a single isomer and not a mixture of epimers.⁷ Reduction of the

benzene-chloroform (3:1) gave a pale yellow gum which yielded *calonecetrin* (3 α ,15-*diacetoxy*-12,13-*epoxytrichothec-9-ene*) (1) (3.78 g) as prisms, m.p. 83–85° (from ether-light petroleum), $[\alpha]_D^{27} +14.6^\circ$ (*c* 1.0 in CHCl₃) (Found: C, 64.8; H, 7.3. C₁₉H₂₆O₆ requires C, 65.1; H, 7.5%); ν_{\max} 1745, 1725, 1240, and 965 cm⁻¹. Elution with benzene-chloroform (1:1) gave a solid which yielded 15-*deacetylcalonecetrin* (3 α -*acetoxy*-12,13-*epoxytrichothec-9-en-15-ol*) (2) (880 mg) as prisms, m.p. 184–186° (from ether-light petroleum), $[\alpha]_D^{27} +11.2^\circ$ (*c* 1.0 in CHCl₃) (Found: C, 66.2; H, 7.9. C₁₇H₂₄O₅ requires C, 66.2; H, 7.85%); ν_{\max} 3550, 1740, 1235, and 960 cm⁻¹. Elution with chloroform gave a pale

Chemical shifts (τ values) of protons in calonecetrin and related compounds^a

Compound	2-H	3-H	10-H ^b	11-H	13-H	14-H	15-H	16-H ^c	Miscellaneous
(1)	6.25(d) (5)	4.72(m)	4.53(d)	ca. 6	7.14(d), 6.90(d) (4)	9.17(s)	6.18(d), 5.88(d) (12)	8.27(s)	OAc 7.89(s), 7.97(s)
(2)	6.27(d) (5)	4.82(m)	4.53(d) (5)	6.02(d) (5)	7.14(d), 6.90(d) (4)	9.08(s)	6.58(d), 6.30(d) (12)	8.27(s)	OAc 7.91 (s), OH 8.45
(3)	6.54(d) (5)	5.60(m)	4.50(d) (5)	5.92(d) (5)	7.18(d), 6.94(d) (4)	9.12(s)	6.53(d), 6.30(d) (12)	8.27(s)	OH 7.4, ca. 8.4
(4)	6.48(d) (5)	5.60(m)	4.50(d) (5)	5.82(d) (5)	7.14(d), 6.90(d) (4)	9.18(s)	6.17(d), 5.86(d) (12)	8.28(s)	OAc 7.94(s), OH 7.7
(5)	6.63(s)		4.55(d) (5)	ca. 6.08	6.95(d), 6.77(d) (4)	9.01(s)	6.10(d), 5.89(d) (13)	8.28(s)	OAc 7.95(s), 4-H ₂ 7.65(d), 7.11(d) (19)
(7)	6.69(s)		4.58(d) (6)	6.08(d) (6)	6.98(d), 6.84(d) (4)	8.96(s)	6.42(d), 6.38(d) (3)	8.28(s)	4-H ₂ 7.74(d), 6.92(d) (19)
(8)	6.25(d) (5)	4.85(m)	4.56(d) (5)	5.98(d)	7.17(d), 6.93(d) (4)	9.17(s)	6.18(d), 5.92(d) (14)	8.30(s)	Me 8.84(t) (7), OAc 7.97(s)
(9)	6.27(d) (4)	4.90(m)	4.60(d) (5)	6.00(d)	7.18(d), 6.95(d) (4)	9.18(s)	6.20(d), 5.96(d) (12)	8.30(s)	Me 9.04(t) (7), OAc 7.99(s)
(10)	6.31(d) (5)	4.95(m)		5.97	7.20(d), 6.95(d) (4)	9.26(s)	6.02(s)	9.15(d) (6)	OAc 8.00(s), 7.98(s)
(11)	6.18(d) (4)	4.70(m)	4.63(d) (4)	6.12(d) (4)	8.54(s)	8.94(s)	6.26(d), 5.90(d) (12)	8.32(s)	OAc 7.98(s), 7.94(s)
(12)	6.33(d) (4)	ca. 5(m)		5.96(d) (4)		8.88(s)	5.96(d), 5.60(d) (12)	8.80(s)	OAc 7.93(s), 7.95(s), OH 7.8–8.4 (2)

^a The figures in parentheses are the coupling constants (Hz). ^b Appears as a broad signal due to coupling with 16-H₃. ^c When the 9,10-double bond is present this signal is broadened owing to coupling with 10-H.

diol (3) with lithium aluminium hydride and acetylation of the product gave the tertiary alcohol (11). Treatment of calonecetrin with hot water gave the hydrate (12) (*cf.* diacetoxyscirpenol⁵ and verrucarol⁷). The propionyl (8) and butyryl (9) derivatives were prepared from 3-deacetylcalonecetrin (4).

EXPERIMENTAL

I.r. spectra were measured for Nujol mulls. N.m.r. spectra were measured for solutions in deuteriochloroform on either a Varian A 60 or a Varian HA 100 spectrometer. Hopkin and Williams silica gel MFC was used for column chromatography, and t.l.c. was carried out on Merck silica gel GF. Light petroleum used had b.p. 60–80°.

Isolation of the Metabolites.—*Calonecetrin nivalis* Schaffnit (C.M.I. 14764, no. 1731 in our collection) was grown as surface culture for 21 days in Thompson bottles each containing 1 l of Czapek Dox medium. The culture filtrate (70 l) was extracted at its natural pH (7) with ethyl acetate (1 × 14 l, 2 × 7 l) to give a brown gum (13.8 g) which was chromatographed on silica gel (800 g). Elution with

yellow gum which crystallised from ether-light petroleum to give culmorin (50 mg) as prisms, m.p. 178–179° (lit.³ m.p. 174–175°), identified by its i.r., mass, and n.m.r. spectra. Intermediate gummy fractions, which t.l.c. showed to be mixtures, were discarded.

3,15-Dideacetylcalonecetrin (12,13-*Diepoxytrichothec-9-ene-3 α ,15-diol*) (3).—(a) *From calonecetrin* (1). A solution of calonecetrin (400 mg) in methanol (10 ml) was added to 0.3N-sodium hydroxide (12 ml). The mixture was set aside at room temperature for 2 h, diluted with water (150 ml), and extracted with chloroform (5 × 20 ml). The extracts were dried (Na₂SO₄) and evaporated to give a gum which crystallised from ether to give 3,15-*dideacetylcalonecetrin* (3) (181 mg), m.p. 166–167° (Found: C, 67.1; H, 8.1. C₁₇H₂₂O₄ requires C, 67.6; H, 8.3%); ν_{\max} 3450, 3400, and 1680 cm⁻¹.

(b) *From 15-deacetylcalonecetrin* (2). A solution of 15-deacetylcalonecetrin (200 mg) in methanol (5 ml) was added to 0.1N-sodium hydroxide (5 ml). The mixture was set aside at room temperature for 10 min, diluted with water (100 ml), and extracted with ether (5 × 50 ml). The extracts

⁶ B. K. Tidd, *J. Chem. Soc. (C)*, 1967, 218.

⁵ H. P. Sigg, R. Mauli, E. Flury, and D. Hauser, *Helv. Chim. Acta*, 1965, **48**, 962.

⁷ J. Gutzwiller, R. Mauli, H. P. Sigg, and Ch. Tamm, *Helv. Chim. Acta*, 1964, **47**, 2234.

were dried (Na_2SO_4) and evaporated to give a gum which crystallised from ether to give 3,15-dideacetylcalonecetrin (3) (78 mg), m.p. 166—167°.

3-Deacetylcalonecetrin (15-Acetoxy-12,13-epoxytrichothec-9-en-3 α -ol) (4).—A solution of calonecetrin (480 mg) in methanol (10 ml) was added to 0.1N-sodium hydroxide (10 ml) and set aside at room temperature for 20 min. The mixture was diluted with water (150 ml) and extracted with ether (4×20 ml). The extracts were dried (Na_2SO_4) and evaporated to give a gum which crystallised from chloroform-light petroleum to give 3-deacetylcalonecetrin (4) (265 mg) as prisms, m.p. 144—145° (Found: C, 65.8; H, 7.7. $\text{C}_{17}\text{H}_{24}\text{O}_5$ requires C, 66.2; H, 7.85%); ν_{max} 3450, 1735, 1660, and 1240 cm^{-1} .

15-Acetoxy-12,13-epoxytrichothec-9-en-3-one (5).—A solution of 3-deacetylcalonecetrin (4) (95 mg) in dry toluene (25 ml) was heated under reflux for 2 h with silver carbonate-Celite reagent (2.5 g).⁴ The suspension was filtered through Celite and the filtrate was evaporated to give a gum which crystallised from chloroform-light petroleum to give the ketone (5) (66 mg) as prisms, m.p. 169—171° (Found: C, 66.2; H, 7.2. Calc. for $\text{C}_{17}\text{H}_{22}\text{O}_5$: C, 66.6; H, 7.2%); ν_{max} 1755, 1745, 1680, and 1240 cm^{-1} (lit.,⁵ m.p. 170—171°, ν_{max} 1745, 1725, 1670, 1235, and 960 cm^{-1}).

15-Hydroxy-12,13-epoxytrichothec-9-en-3-one (7).—A solution of the acetoxy-ketone (5) (100 mg) in methanol (15 ml) was added to 0.1N-sodium hydroxide (10 ml). The mixture was set aside at room temperature for 2 h, diluted with water (75 ml), and extracted with chloroform (3×50 ml). The extracts were dried (Na_2SO_4) and evaporated to give a gum which on crystallisation from ether gave the hydroxy-ketone (7) (76 mg) as needles, m.p. 167—169° (Found: C, 67.7; H, 7.9. Calc. for $\text{C}_{15}\text{H}_{20}\text{O}_4$: C, 68.1; H, 7.7%); ν_{max} 3500, 1750, and 1680 cm^{-1} , identical with material prepared from diacetoxyscirpenol by a literature route.⁵

9,10-Dihydrocalonecetrin (3 α ,15-Diacetoxy-12,13-epoxytrichothecane) (10).—A solution of calonecetrin (1) (200 mg) in ethanol (25 ml) was shaken with 5% palladium-charcoal (20 mg) in hydrogen for 16 h. Filtration and evaporation gave crystals which afforded 9,10-dihydrocalonecetrin (11) (121 mg) as needles, m.p. 93—95° (from light petroleum) (Found: C, 65.0; H, 7.7. $\text{C}_{19}\text{H}_{28}\text{O}_6$ requires C, 64.75; H, 8.0%); ν_{max} 1740 and 1240 cm^{-1} . Concentration of the mother liquors yielded a second crop (63 mg).

3,15-Diacetoxytrichothec-9-en-12-ol (11).—Lithium aluminium hydride (1 g) was added to a solution of dideacetylcalonecetrin (3) (1 g) in anhydrous ether (300 ml) and the mixture was stirred and heated under reflux for 24 h. Aqueous ammonium chloride (10%; 100 ml) was added to the cooled mixture, the ether layer was separated, and the

aqueous layer was extracted continuously for 16 h with ethyl acetate. The organic extracts were dried (Na_2SO_4) and evaporated; the residue (850 mg) was dissolved in pyridine (15 ml) and treated with acetic anhydride (10 ml). After 45 min the solution was poured on crushed ice and extracted with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated to give a gum which crystallised from ether to give 3,15-diacetoxytrichothec-9-en-12-ol (11) (276 mg) as prisms, m.p. 165—167° (Found: C, 64.9; H, 7.9. $\text{C}_{19}\text{H}_{28}\text{O}_6$ requires C, 64.7; H, 8.0%); ν_{max} 3400, 1740, 1720, 1250, and 1235 cm^{-1} .

Preparative t.l.c. in chloroform-methanol-formic acid (90 : 5 : 5) of the mother liquor yielded more tertiary alcohol (11) (216 mg), starting material (3) (115 mg), and an unidentified polar compound (81 mg) which could not be crystallised.

Calonecetrin Hydrate (12).—A solution of calonecetrin (1) (200 mg) in chloroform (15 ml) was added to water (50 ml); the chloroform was evaporated off and the resulting aqueous solution was heated on a steam-bath for 6 h. The cooled solution was extracted with ethyl acetate (3×10 ml) and the extract was dried (Na_2SO_4) and evaporated. The crystalline residue gave calonecetrin hydrate (12) (170 mg) as needles, m.p. 168—169° (from chloroform-light petroleum) (Found: C, 61.8; H, 7.4. $\text{C}_{19}\text{H}_{28}\text{O}_7$ requires C, 61.7; H, 7.7%); ν_{max} 3420, 3370, 1740, 1720, and 1250 cm^{-1} .

15-Acetoxy-12,13-epoxy-3 α -propionyloxytrichothec-9-ene (8).—A solution of 3-deacetylcalonecetrin (3) (100 mg) in pyridine (3 ml) was treated with propionic anhydride (2.5 ml), set aside at room temperature for 3 h, diluted with water, and extracted with ether (3×15 ml). The extract was dried (Na_2SO_4) and evaporated; the crystalline residue gave the propionyl derivative (8) (75 mg) as prisms, m.p. 79—81° (from light petroleum) (Found: C, 66.2; H, 7.7. $\text{C}_{20}\text{H}_{28}\text{O}_6$ requires C, 65.9; H, 7.7%); ν_{max} 1740, 1680, and 1250 cm^{-1} .

15-Acetoxy-3 α -butyryloxy-12,13-epoxytrichothec-9-ene (9).—A solution of 3-deacetylcalonecetrin (3) in pyridine (5 ml) was treated with butyric anhydride (3.5 ml), set aside at room temperature for 3 h, diluted with water (100 ml), and extracted with chloroform (4×50 ml). The extract was dried (Na_2SO_4) and evaporated to give an oil which contained butyric acid. The oil was chromatographed on silica gel (6 g); elution with benzene-chloroform (1 : 1) gave a gum which, after being dried under vacuum to remove the last traces of butyric acid, crystallised and gave the butyryl derivative (9) (85 mg) as prisms, m.p. 69—70° [from light petroleum (b.p. 40—60°)] (Found: C, 66.8; H, 8.1. $\text{C}_{21}\text{H}_{30}\text{O}_6$ requires C, 66.7; H, 7.9%); ν_{max} 1740, 1680, and 1240 cm^{-1} .

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