Phytotoxic Compounds Produced by *Fusarium equiseti*. Part 10.[1] The Preparation and Rearrangement of Diacetylneosolaniol 9β,10β-Epoxide

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Under conditions where trichothecodiol 9β,10β-epoxide rearranges to an 8α,9α:10β,13-diepoxytrichothecan-12-ol, diacetylneosolaniol 9β,10β-epoxide gives a 9α,15:12,13-diepoxytrichothecan-10β-ol. Some by-products formed during the allylic bromination and allylic oxidation of diacetoxyscirpenol have been identified. One of them is a 3α,11α:12,13-diepoxytrichothecene.

Previous work[1] on the chemistry of trichothecene 9β,10β:12,13-diepoxides has shown that intramolecular attack on the 9β,10β-epoxide from oxygen anions at positions 8α and 15 takes precedence over external nucleophilic attack. Thus, with dilute aqueous sodium hydroxide, the 9β,10β-epoxide (**2**) of trichothecodiol (**1**) gives the 8α,9α:10β,13-diepoxy-12-ol (**3**) (together with isotrichothecolone) whilst the epoxide (**5**) of scirpentriol (**4**; R = H) yields the 9α,15:12,13-diepoxy-10β-ol (**6**). T2-Tetraol (**7**; $R^1 = R^2 = R^3 = H$),[2] the polyol from which the important mycotoxin esters neosolaniol (**7**; $R^1 = R^3 = H$, R^2 = Ac) and T2-toxin (**7**; $R^1 = Me_2CHCH_2CO$, R^2 = Ac, R^3 = H) are derived, contains hydroxyl functions at both positions 8α and 15, offering a choice of reaction path. When the 9β,10β-epoxide (**8**; R = Ac) was treated with 1M sodium hydroxide at 100 °C, conditions favourable to the formation of an 8α,9α:10β,13-diepoxide,[1] the product was the 9α,15:12,13-diepoxy-10β-ol (**9**), as shown by the NMR spectrum (Table) (w coupling $J_{7\beta,15}$ 3.2 Hz) of the isolated tetra-acetate (**9**; R = Ac): no 8α,9α:10β,13-diepoxy-12-ol was obtained.

Although epoxidation of trichothecodiol (**1**) was straightforward[1] and the derived β-epoxide (**2**) was, unexpectedly, the sole product, epoxidation of neosolaniol (**7**; $R^1 = R^3 = H$, R^2 = Ac) was complex. Accordingly, attention was transferred to a derivative in which the 8α-hydroxy group was protected and the 3α,8α-diacetate (**7**; $R^1 = R^2 = R^3$ = Ac) was a convenient choice. This derivative gave only one product, shown by the NMR spectrum ($J_{10,11}$ 5.5 Hz) to be the β-epoxide (**8**; R = Ac). Trichothecene esters are hydrolysed so readily[2] that use of the tetra-acetate (**8**; R = Ac) in a base-catalysed rearrangement presented no drawback.

The most direct route to the acetate (**7**; $R^1 = R^2 = R^3$ = Ac) from the readily available diacetoxyscirpenol (**4**; R = Ac) involved allylic bromination with *N*-bromosuccinimide[3,4] followed by acetolysis of the 8β-bromo derivative (**10**; R = Br) with fused sodium acetate in boiling acetic anhydride. Separation of the resulting 8-epimeric tetra-acetates by column chromatography presented no difficulty.

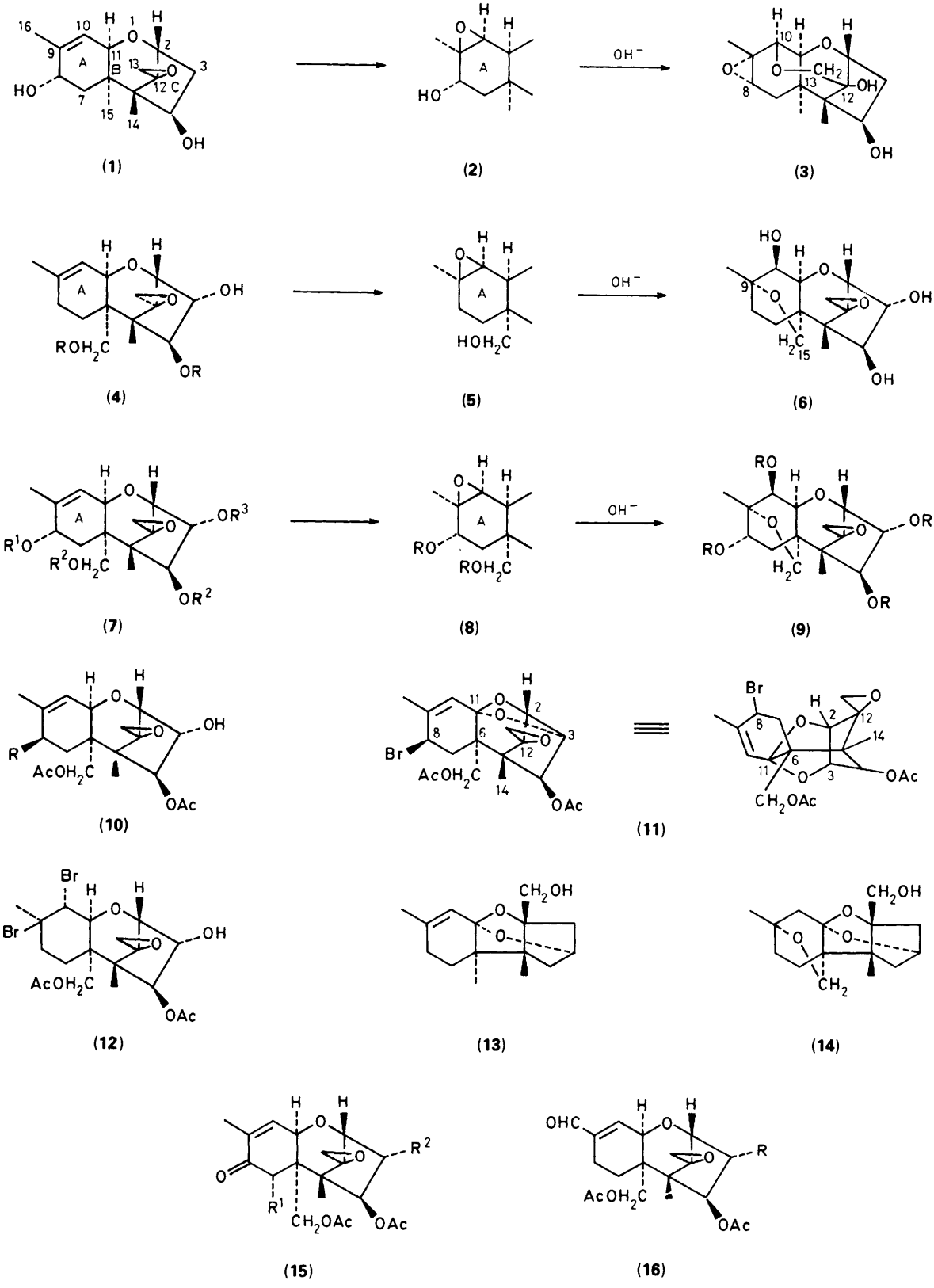
The formation of a number of unidentified by-products during the allylic bromination of diacetoxyscirpenol (**4**; R = Ac) was reported.[4] Two of these have now been identified as the 8β-bromo-3α,11α-epoxide (**11**) and the known[4] 9β,10α-dibromo derivative (**12**). The formation of some dibromide in reactions with *N*-bromosuccinimide is not unusual. The bromo compound (**11**) had the composition $C_{19}H_{23}BrO_7$ and showed no OH absorption in the IR spectrum. The NMR spectrum (Table) revealed the absence of H-11 and only allylic couplings to H-10. The coupling constants $J_{7\alpha,8}$ 6.9 Hz and $J_{7\beta,8}$ 9.9 Hz were consistent with ring A approximating to the normal half-chair conformation[1] with the 8-bromo substituent in the β-configuration.[1] It was concluded that an additional ether linkage had been formed between positions 3 and 11: there is no evidence to show whether this is formed directly, after abstraction of H•; or by the elimination of HBr following bromination at position 11. Apotrichothecene-3α,11α-epoxides are known, *e.g.* (**13**)[5] and the apotrichothecane sporol (**14**),[6] but trichothecene-3α,11α-epoxides have not hitherto been recorded. Molecular models of the diepoxide (**11**) show that, resulting from the additional strain imposed on ring C, the dihedral angle between H-3β and H-4α is *ca.* 90° and, in consequence, $J_{3\beta,4\alpha}$ = 0 Hz.

Trichothecene chemistry is dominated by intramolecular reactions[1,2] and the formation of the diepoxide (**11**) provides one more example of this capability.

Oxidation with selenium(IV) oxide provides an alternative route from diacetoxyscirpenol to neosolaniol.[3] As expected, by analogy with parallel work in the verrucarol series,[7] selenium(IV) oxide oxidation of diacetoxyscirpenol gave, in addition to the 8β-hydroxy derivative (**10**; R = OH) (39%),[3] a substantial fraction (38%) containing α,β-unsaturated carbonyl compounds. The chromatographic separation of this fraction into its components was difficult, but some 4β,15-diacetylnivalenol (**15**; $R^1 = R^2$ = OH)[8] was obtained in addition to the 16-oxo- (**16**; R = OH) (δ 9.55, H-16) and the known 8-oxo- (**15**; R^1 = H, R^2 = OH)[3] derivatives of diacetoxyscirpenol. A mixture of the 8-oxo (**15**; $R^1 = R^2$ = H) and 16-oxo (**16**; R = H) compounds was obtained from diacetylverrucarol under similar conditions[7] and hydroxylation of the macrocyclic verrucarins at position 16, as well as position 8, has been reported.[9] Diacetylnivalenol presumably results from the use of excess selenium(IV) oxide.

Experimental

M.p.s were taken on a Kofler hot stage apparatus and are corrected. Unless otherwise stated, IR spectra were determined on mulls in Nujol. UV spectra were recorded from solutions in MeOH. ^{1}H NMR spectra were obtained at 360 MHz in $CDCl_3$ with $SiMe_4$ as internal standard. Molecular weights were taken from the mass spectra. NH_3 was used to obtain chemical ionisation mass spectra (CIMS). In analytical TLC, Merck silica gel 60 F_{254} was used with chloroform–methanol (49:1; solvent A) or (9:1; solvent B). Spots were visualised in UV light, in iodine vapour, or by heating after spraying with methanolic sulphuric acid, as appropriate. Merck silica gel 7734 was used for column chromatography. Acetylations were carried out at



room temperature in pyridine with acetic anhydride. Light petroleum had b.p. 60–80 °C. Identifications were confirmed by comparison of the IR spectra.

3α,4β,8α,15-*Tetra-acetoxy*-9β,10β:12,13-*diepoxytrichothecane* (**8**; R = Ac).—Diacetylneosolaniol (3α,4β,8α,15-tetra-acetoxy-12,13-epoxytrichothec-9-ene) (**7**; $R^1 = R^2 = R^3 =$ Ac) (28 mg) and 3-chloroperoxybenzoic acid (20 mg) in dichloromethane (2 ml) were stirred at room temperature and the course of the reaction was monitored by analytical TLC (A). After 7 days, more 3-chloroperoxybenzoic acid (14 mg) was added and the reaction was allowed to continue for a further

Table. ^{1}H NMR resonances (δ, *J* Hz in parentheses[a]) for the 9β,10β-epoxide (**8**; R = Ac) and its relatives.

Position	2	3	4	7α	7β	8	10	11	13	14	15	16	Ac	OH
(**8**; R = Ac)	3.96d (4.7)	5.19dd (4.7, 3.3)	5.72d (3.3)	1.68ddd (14.4, 2.1, 0.9)	2.14dd (14.4, 4.5)	5.27dt (4.5, 0.9)	3.32dd (5.5, 0.9)	4.18dd (5.5, 2.1)	3.12d **AB** 2.76d (3.9)	0.68s	4.40d **AB** 4.07d (12.5)	1.36s	2.04 2.10 2.11 2.16	—
(**9**; R = Ac)	3.92d (4.9)	5.15dd (4.9, 3.4)	5.60d (3.4)	1.45dd (14.5, 2.6)	2.50ddd (14.5, 8.6, 3.2)	5.09d (8.6)	5.14d (8.4)	4.23dd (8.4, 2.6)	3.01d **AB** 2.76d (3.9)	0.58s	4.01dd (9.6, 3.2) 3.81d (9.6)	1.14s	2.07 2.11 2.16 2.16	—
(**11**)	4.24d (4.3)	4.69d (4.3)	5.51s	2.59dd (13.3, 6.9)	2.70dd (13.3, 9.9)	4.53dd (9.9, 6.9)	5.54t (1.4)	—	3.07d **AB** 2.79d (4.1)	0.81s	4.37d **AB** 4.16d (13.0)	1.95d — (0.8)	2.08 2.10	—
(**16**; R = OH)	3.76d (4.9)	4.23dd (4.9, 3.0)	5.12d (3.0)	*b*	*b*	*b*	6.73dd (5.3, 2.0)	4.41d (5.3)	3.08d **AB** 2.82d (4.0)	0.87s	4.20d **AB** 3.82d (12.7)	9.55s	2.04 2.17	3.38

[a] First-order approximations from line separations. [b] Not first order.

7 days when all the starting material (R_F 0.58) had been consumed. After being washed with sodium hydrogen carbonate and water, recovery afforded a gum, R_F 0.51 (35 mg) which was dissolved in ethyl acetate–light petroleum (1:1) and chromatographed on a column of silica gel (3 g, 10 × 1.0 cm) made up in light petroleum. Elution with ethyl acetate–light petroleum gave: (i) 50 ml (2:1), 5 mg, discarded and (ii) 30 ml (1:1), gum, 28 mg which crystallised from ethyl acetate–light petroleum in prisms, m.p. 232–235 °C (decomp.) (15 mg) of the *diepoxide* (**8**; R = Ac) (Found: C, 57.1; H, 6.3; MNH$_4^+$, 500. $C_{23}H_{30}O_{11}$ requires: C, 57.3; H, 6.3%; M, 482); ν_{max} 1 734 cm^{-1}.

3α,4β,8α,10β-*Tetra-acetoxy*-9α,15:12,13-*diepoxytrichothecane* (**9**; R = Ac).—The diepoxide (**8**; R = Ac) (8 mg) in 1M sodium hydroxide (1.0 ml) was heated at 100 °C for 4 h. The solution was neutralised with hydrochloric acid and continuously extracted with chloroform for 3 days. The amorphous solid product (6 mg) was acetylated during 4 days to give a gum (8 mg) which was dissolved in ethyl acetate–light petroleum (1:1) and chromatographed on a column (7 × 1.0 cm) of silica gel (2 g) with monitoring by TLC (A). Elution with ethyl acetate–light petroleum (2:1) gave, after a fore-run (20 ml), a fraction R_F 0.57, (8 mg, 30 ml) which crystallised from ethyl acetate–light petroleum in large prisms of the *diepoxide* (**9**; R = Ac), m.p. 172–173 °C, [Found: C, 57.3; H, 6.3; MNH$_4^+$, 500. $C_{23}H_{30}O_{11}$ requires: C, 57.3; H, 6.3%; M, 482); ν_{max} (OH absent) 1 738 cm^{-1}.

Further elution of the column with ethyl acetate–light petroleum (20 ml, 1:1) gave a trace (<1 mg) of material, R_F 0.37, λ_{max} 220 nm which was not identified.

3α,4β,8α,15-*Tetra-acetoxy*-12,13-*epoxytrichothec*-9-*ene* (**7**; R^1 = R^2 = R^3 = Ac).—4β,15-Diacetoxy-8β-bromo-12,13-epoxytrichothec-9-en-3α-ol (**10**; R = Br) (150 mg)[3,4] and fused sodium acetate (450 mg) were heated under reflux with acetic anhydride (4.5 ml) for 2 h. The mixture was poured onto crushed ice, and the resulting solution was neutralised with sodium hydrogen carbonate and extracted with chloroform. The recovered gum (154 mg) in light petroleum–ethyl acetate (2:1) was chromatographed on a column (16 × 1.0 cm) of silica gel (6 g) made up in light petroleum. Fractional elution (4 ml fractions) with light petroleum–ethyl acetate (2:1) monitored by analytical TLC (A) gave the following gummy fractions: (i) 40 ml, R_F 0.75, 11 mg discarded; (ii) 20 ml, R_F 0.63, 84 mg; and (iii) 32 ml, R_F 0.58, 59 mg.

Crystallisation of fraction (ii) from ethyl acetate–light petroleum gave prisms (47 mg, 30%) m.p. 138–140 °C (lit.,[4] m.p. 137–138 °C) of 3α,4β,8β,15-tetra-acetoxy-12,13-epoxytrichothec-9-ene, also obtained (m.p. 143 °C) by acetylation of the diol (**10**; R = OH).

Crystallisation of fraction (iii) from ethyl acetate–light petroleum gave prisms (39 mg, 25%), m.p. 176–178 °C (lit.,[4] m.p. 180–182 °C) of 3α,4β,8α,15-tetra-acetoxy-12,13-epoxytrichothec-9-ene (**7**; R^1 = R^2 = R^3 = Ac), also obtained (m.p. 177–179 °C) by acetylation of neosolaniol.

Attempted Epoxidation of Neosolaniol.—Neosolaniol (**7**; R^1 = R^3 = H, R^2 = Ac) (10 mg), prepared by a literature method,[3] and 3-chloroperoxybenzoic acid (7 mg) in dichloromethane (3 ml) were stirred at 25 °C and the course of the reaction was monitored by analytical TLC (B). After 24 h, more 3-chloroperoxybenzoic acid (7 mg) was added and the reaction was continued for a further 2 days without obviously further decreasing the amount of starting material (R_F 0.50) remaining. Recovery, after washing with sodium hydrogen carbonate and water, gave a gum (6 mg) which showed spots at R_F 0.62, 0.55, 0.50, 0.41 and 0.35. The gum was chromatographed on a column (6 × 1.0 cm) of silica gel (2 g) made up in dichloromethane and eluted with dichloromethane–methanol (200:1). All the materials in the range R_F 0.62–0.35 were separately eluted, some fractions being of negligible weight, but no crystalline products were obtained.

Bromination of Diacetoxyscirpenol with N-*Bromosuccinimide.*—Diacetoxyscirpenol (**4**; R = Ac) (366 mg) and *N*-bromosuccinimide (267 mg, 1.5 mmol) were stirred at room temperature under nitrogen in dichloromethane (10 ml) in the presence of powdered 3 Å molecular sieves (300 mg) whilst being illuminated by a tungsten filament lamp. The solution became orange (1 h) and then colourless (3 h). After 4 h, when no starting material remained (TLC), the mixture was filtered and the filtrate was washed with water. Recovery afforded a foam (500 mg) which was dissolved in ethyl acetate–light petroleum (1:1) and chromatographed on a column (26 × 1.8 cm) of silica gel (30 g) made up in light petroleum. Elution with ethyl acetate–light petroleum (1:1), monitored by analytical TLC (B), gave, after a forerun (100 ml), the following gummy fractions: (i) 50 ml, R_F 0.67, 63 mg; (ii) 20 ml, R_F 0.45, 106 mg; (iii) 10 ml, interband, 64 mg; and (iv) 50 ml, R_F 0.38. 213 mg, 48% identified by the NMR spectrum[3,4] as the 8β-bromo derivative (**10**; R = Br).

Fraction (i) was rechromatographed on silica gel (4 g, 12 × 1.0 cm) to give 4β,15-*diacetoxy*-8β-*bromo*-3α,11α:12,13-*diepoxytrichothec*-9-*ene* (**11**) as a foam [Found: C, 51.4; H, 5.2; M^+, 442. $C_{19}H_{23}BrO_7$ requires: C, 51.5; H, 5.2%; M, 442 (Br = 79)]. ν_{max} (OH absent) 1 747 (1 750 cm^{-1} in CH_2Cl_2).

Fraction (ii) was rechromatographed twice on silica gel (6 g, 16 × 1.0 cm) to give the 9β,10α-dibromo derivative (**12**), identified by its NMR spectrum.[4]

Oxidation of Diacetoxyscirpenol with Selenium(IV) *Oxide.*—Diacetoxyscirpenol (**4**; R = Ac) (732 mg), dissolved in dioxane (30 ml) and water (1 ml) was heated under reflux with selenium(IV) oxide (225 mg) for 22 h. The solution was decanted from the precipitated material and the solvent removed by distillation under reduced pressure. The residue was extracted with dichloromethane and the concentrated extract was chromatographed on a column (20 × 1.8 cm) of silica gel (23 g) made up in dichloromethane. Elution with dichloromethane–methanol (50:1) monitored by TLC (B) gave the following fractions: (i) 150 ml, 3 mg, discarded; (ii) 50 ml, R_F 0.6–0.5, 240 mg, 32%; (iii) 50 ml, R_F 0.50, 47 mg, 6%; and (iv) 200 ml, R_F 0.28, 303 mg, 39%. Further elution with dichloromethane–methanol (25:1, 50 ml) gave intractable material, R_F 0.18–0.15 (33 mg).

Fraction (iv) crystallised from dichloromethane in prisms, m.p. 112–115 °C (lit.,[3] m.p. 114–116 °C) of the 8β-ol (**10**; R = OH).

Fraction (iii) was shown by its NMR spectrum to consist of 4β,15-*diacetoxy*-12,13-*epoxy*-3α-*hydroxytrichothec*-9-*en*-16-*one* (**16**; R = OH) and was obtained as an amorphous solid, m.p. 60–85 °C (Found: C, 59.9; H, 6.4; MNH$_4^+$, 398. $C_{19}H_{24}O_8$ requires: C, 60.0; H, 6.4%, M, 380); ν_{max} (CH_2Cl_2) 3 570, 2 810, 1 740, 1 725, and 1 690 cm^{-1}; λ_{max} 224 nm (log ε 3.75).

Rechromatography of a portion (165 mg) of fraction (ii) on silica gel (5 g, 14 × 1.0 cm) and elution with dichloromethane (100 ml) followed by dichloromethane–methanol (200:1) gave: (i) 50 ml, 1 mg, discarded; (ii) 6 ml, R_F 0.62, 5 mg, which crystallised from ethyl acetate–light petroleum in prisms, m.p. 130–133 °C (lit.,[8] m.p. 135–136 °C) of 4β,15-diacetylnivalenol (**15**; R^1 = R^2 = OH); (iii) 50 ml, R_F 0.58, 12 mg, foam, identified by its NMR spectrum as the 8-oxo compound (**15**; R^1 = H, R^2 = OH)[3]; and (iv) 100 ml, R_F 0.58–0.50, 80 mg, shown by its NMR spectrum to consist of a mixture of the 8-oxo (**15**; R^1 = H, R^2 = OH) and 16-oxo (**16**; R = OH) compounds. Further elution of the column with dichloromethane–methanol

(100:1) furnished fraction (v) 100 ml, R_F 0.50, 55 mg, shown by its NMR spectrum to consist mainly of the 16-oxo compound (**16**; R = OH).

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