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SYNTHESIS AND NMR ANALYSIS OF NEW NATURAL TRICHOTHECENES[1]

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The fungal species *Fusarium graminearum*, *Fusarium moniliforme*, and *Fusarium sporotrichioides* commonly infest grains, which can lead to the contamination of crops with a variety of mycotoxins. The major metabolite produced on culturing a Canadian isolate of *F. sporotrichioides* was T-2 [**4a**] (Table 1); in addition, some 16 minor compounds were isolated (1). The ^{1}H-nmr and mass spectral data for three of these minor metabolites (≤1 mg) suggested that they were analogs of T-2 toxin [**4c**, **4d**, and **4e**], differing only in substitution at C-8. The production of T-2 and other trichothecenes on a large scale for biological assay enabled their use as templates for chemical modification and the characterization of **4c**, **4d**, and **4e**.

The synthesis of the three analogs is shown in Figure 1. Neosolaniol [**1**] was acylated with the appropriate acid

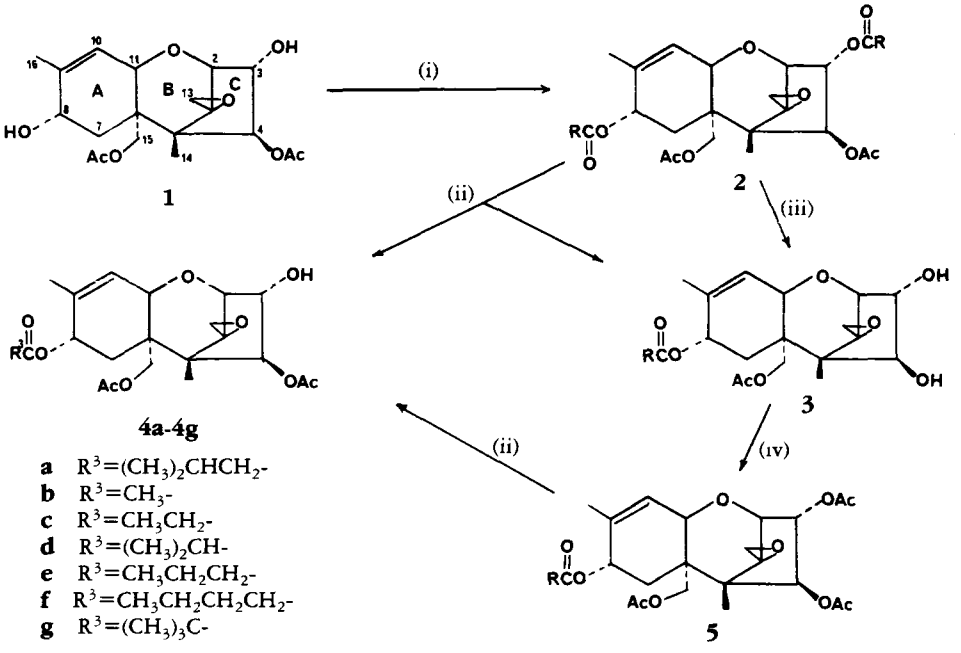


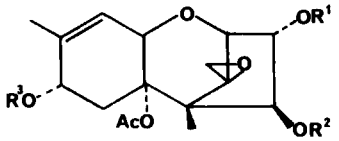
FIGURE 1. (i) RCOCl, pyridine, CH_2Cl_2, room temperature, overnight; (ii) HCl/MeOH (1:100), room temperature, overnight; (iii) NH_4OH/MeOH, room temperature, 4 h; (iv) AcCl, pyridine, CH_2Cl_2, room temperature, overnight

chloride and pyridine in CH_2Cl_2 (2) to yield the intermediates **2**.[2] In addition to the desired propionyl, butyryl, and isobutyryl groups, three other acyl groups, i.e., acetyl, valeryl, and trimethylacetyl, were used to complement the series. For compounds with straight-chain acyl groups, overnight

[1]PRC contribution no. 1038.

[2]For the sake of simplicity, compounds were numbered in series. For instance, **2d**, **3d**, **4d**, and **5d** all have an isobutyryl substituent at C-8 and are part of the same synthetic sequence. As a consequence, some numbers are not found in the data because the corresponding compounds were not made; e.g., **2a** corresponds to 3-isovaleryl-T-2, which was of no synthetic use.

TABLE 1. 250 MHz ^{1}H NMR-Data for the Neosolaniol Derivatives Synthesized[a]



mult.: *J* Hz: #	R^1	R^2	R^3	4 d 3	7α dt 15, 1.5	7β dd 15, 5.5	11 d 6	15a d 12.5	15b d 12.5	16 bs	Acetates	R^3 (*↦R^2) (+↦R^1)
2c	Prop	Ac	Prop	5.87	1.90	2.33	4.18	4.06	4.34	1.71	2.03, 2.07	1.11 (t, 7), 2.25 (q, 7), 2.27 (q, 7) +: 1.16 (t, 7), 2.42 (q, 7)
2d	iBut	Ac	iBut	6.00	1.77	2.37	4.29	4.05	4.35	1.71	2.06, 2.07	1.14 (d, 7), 1.15 (d, 7), 2.47 (sep, 7) +: 1.19 (d, 7), 1.20 (d, 7), 2.65 (sep, 7)
2e	But	Ac	But	5.89	1.87	2.34	4.19	4.07	4.33	1.72	2.04, 2.07	0.92 (t, 7), 1.62 (sex, 7), 2.22 (t, 7), 2.23−(+.7) +: 0.97 (t, 7), 1.68 (sex, 7), 2.37 (t, 7)
2f	Val	Ac	Val	5.88	1.86	2.32	4.18	4.06	4.32	1.71	2.03, 2.06	
2g	tMAc	Ac	tMAc	6.19	1.63	2.42	4.44	4.01	4.39	1.72	2.07, 2.09	1.19 (s), +: 1.24 (s)
2i	Prop	Prop	iVal	5.91	1.85	2.35	4.21	4.07	4.33	1.72	2.05	0.92 (d, 7), 0.93 (d, 7), 2.1 (m), 2.11 (m) *, +: 1.12 (t, 7), 1.16 (t, 7), 2.37 (q, 7), 2.42 (q, 7).
3a	H	H	iVal	4.36	1.96	2.31	4.13	3.95	4.29	1.71	2.01	0.92 (d, 7), 0.93 (d, 7), −2.0−2.2 (m)
3b	H	H	Ac	4.34	2.03	2.31	4.08	3.94	4.26	1.71	2.01,2.03	
3c	H	H	Prop	4.39	1.97	2.33	4.16	3.98	4.27	1.72	2.02	1.13 (t, 7), 2.26 (q, 7), 2.28 (q, 7)
3d	H	H	iBut	4.45	1.88	2.36	4.22	3.97	4.32	1.72	2.03	1.15 (d, 7), 1.16 (d, 7), 2.46 (sep, 7)
3e	H	H	But	4.37	1.97	2.32	4.14	3.96	4.29	1.72	2.02	0.93 (t, 7), 1.63 (sex, 7), 2.21 (t, 7), 2.22 (t, 7)
3f	H	H	Val	4.37	1.97	2.32	4.16	3.96	4.27	1.72	2.02	0.90 (t, 7) 1.35 (sex, 7), 1.57 (bp, 7), 2.24 (t, 7), 2.25 (t, 7)
4a	H	Ac	iVal	5.28	1.89	2.38	4.32	4.03	4.27	1.73	2.01, 2.12	0.94 (d, 7), 0.95 (d, 7) 2.0 (m), 2.12 (m) +: 3.18 (d, 2.8)

4b	H	Ac	Ac	5.19	1.96	2.33	4.25	4.03	4.27	1.71	2.00, 2.11	1.99 (s)
4c	H	Ac	Prop	5.25	1.92	2.37	4.30	4.03	4.27	1.72	2.00, 2.12	1.12 (t, 7.5), 2.26 (q, 7.5), 2.27 (q, 7.5)
4d	H	Ac	iBut	5.36	1.82	2.41	4.38	4.02	4.31	1.72	2.01, 2.13	1.15 (d, 7), 1.16 (d, 7), 2.47 (sep, 7)
4e	H	Ac	But	5.26	1.90	2.37	4.31	4 03	4.27	1.72	2.01, 2.12	0.93 (t, 7), 1.63 (sex, 7), 2.22 (t, 7), 2.23 (t, 7)
4f	H	Ac	Val	5.26	1.90	2.37	4.31	4.03	4.27	1.72	2.01, 2.12	0.89 (t,7), 1.32 (sex, 7), 1.59 (m), 2.24 (t, 7), 2.25 (t, 7)
4g	H	Ac	tMAc	5.56	1.67	2.45	4.49	3.97	4.37	1.72	2.03, 2.13	1.19 (s)
4h	H	Prop	Ac	5.17	1.98	2.35	4.27	4.04	4.25	1.73	2.02, 2.02	*: 1.15 (t, 7), 2.41 (q, 7)
4i	H	Prop	iVal	5.26	1.88	2.37	4.32	4.03	4.26	1.71	2.00	0.92 (d, 7), 0.93 (d, 7), 2.0-2.2 (m) *: 1.73 (t, 7), 2.39 (q, 7)
5b	Ac	Ac	Ac	5.80	1.95	2.30	4.12	4.08	4.33	1.72	2.00, 2.13	2.03 (s), +: 2.08 (s)
5d	Ac	Ac	iBut	5.96	1.81	2.37	4.24	4.05	4.35	1.73	2.06, 2.14	1.15 (d, 7), 1.16 (d, 7), 2.47 (sep, 7) +: 2.08 (s)
5e	Ac	Ac	But	5.87	1.88	2.33	4.17	4.07	4.33	1.72	2.04, 2.14	0.92 (t, 7), 1.63 (sex, 7), 2.19 (t, 7), 2.22 (t, 7), +: 2.08 (s)
5f	Ac	Ac	Val	5.88	1.89	2.34	4.18	4.07	4.34	1.72	2.05, 2.14	0.89 (t, 7), 1.32 (sex, 7), 1.55 (bp, 7), 2.24 (t, 7), 2.25 (t, 7), +: 2.08 (s)
6g	iBut	Ac	H	5.92	1.84	2.28	4.19	4.27	4.36	1.83	2.01, 2.08	+: 1.23 (s)

[a]Note: Chemical shifts for the other protons were:

for series **2** compounds: 3.84 (d, 4.9 Hz) (pos. 2), 5.13 (dd, 4.9, 3) (3), 5.25 (bd, 5), (8), 5.69 (bd, 6), (10), 2.78 (d, 4), (13a), 3.04 (d, 4), (13b), 0.72 (s) (14)

for series **3** compounds: 3.61 (d, 4.9 Hz), (pos. 2), 4.22 (dd, 4.9, 3) (3), 5.26 (bd, 5) (8), 5.74 (bd, 6), (10), 2.75 (d, 4), (13a), 3.01 (d, 4) (13b), 0.78 (s) (14)

for series **4** compounds: 3.68 (d, 4.9 Hz) (pos. 2), 4.14 (dd, 4.9, 3) (3), 5.26 (bd, 5.5) (8), 5.78 (bd, 6) (10), 2.78 (d, 4), (13a), 3.04 (d, 4) (13b), 0.79 (s) (14)

for series **5** compounds: 3.61 (d, 4.9 Hz) (pos. 2), 4.22 (dd, 4.9, 3) (3), 5.26 (bd, 5) (8), 5.74 (bd, 6) (10), 2.75 (d, 4) (13a), 3.01 (d, 4) (13b), 0.78 (s) (14)

Abbreviations: b: broad, s: singlet, d: doublet, t: triplet, q: quartet, p: pentuplet, sex: sextuplet, sep: septuplet, m: multiplet. Ac=Acetyl, Prop=Propionyl, But=Butyryl, iBut=isoButyryl, tMAc=trimethylacetyl, Val=Valeryl, iVal=isovaleryl.

All chemical shifts ±0.01 ppm; all coupling constants ±0.3 Hz.

hydrolysis with 1% conc. HCl in MeOH yielded a mixture (ca. 2:1) of the desired product **4** and the HT-2 analog **3**. For the branched-chain compounds such as **2d** and **2g**, this mild acid treatment induced hydrolysis at the 4-position instead of the 3-position due to steric hindrance. In this case, hydrolysis was carried through to the diols **3** using equal volumes of MeOH and 2M NH_4OH for 4 h in a modification of a published method (3). Acetylation of these diols followed by acid hydrolysis yielded the desired product mixture of **4** and **3**. In the case of the trimethylacetyl analog **4g**, acetylation was incomplete even after 5 days; however, the product was a mixture of the di- and mono-acylated neosolaniols **2g** and **6g**, respectively, and the desired **4g**, thus eliminating the need for further reaction.

The ^{1}H-nmr data for the various trichothecene intermediates and products **2** to **6** are given in Table 1. The chemical shifts of protons common to each set of compounds (e.g., **2a** to **2i**) are virtually identical, except when the acyl group at C-8 is branched. The extent of branching at the carbon atom α to the carbonyl affects some chemical shifts. For instance, although the H-8 chemical shifts are constant, the resonances of the H-7 methylene protons change significantly. The 7α-proton resonance moves upfield (≤0.25 ppm) while that for the 7β-proton moves only slightly downfield (≤0.08 ppm). The H-4 chemical shifts are also affected, by as much as 0.30 ppm. Both the ^{1}H-nmr and mass spectral data of **4c**, **4d**, and **4e** were identical to those of the compounds isolated from *F. sporotrichioides*, thus confirming the assigned structures.

As further structural proof, the isomer of **4c** having an acetyl moiety at C-8 and a propionyl at C-4 was synthesized by propionylation of **3b** followed by acid hydrolysis to give **4h**. The ^{1}H-nmr data for **4h** differed from those of the naturally occurring **4c**, especially the acetate resonances. The methyl proton resonances for the C-15 acetate in **4** occur at 2.00-2.03 ppm, the C-4 acetate at 2.11-2.14 ppm, and the C-8 acetate at 2.00-2.02 ppm.

Corley *et al.* (4) proposed structure **4i** for a new trichothecene isolated from *F. sporotrichioides* based on nmr data. Its interest lies in the fact that it has a C-4 propionyl moiety. We synthesized this compound by NH_4OH hydrolysis of T-2 toxin to yield **3a**, propionylation to yield **2i**, and, finally, acid hydrolysis to yield the proposed compound **4i**. The nmr spectral data indicated that the assigned structure was correct.

The ^{13}C-nmr spectra of all new T-2 analogs **4b-4f** had chemical shifts virtually identical to those of T-2 except for the 8-acyl moieties which were: **4b**: 20.9, 170.7 (C=O); **4c**: 9.0, 27.7, 174.1 (C=O); **4d**: 18.5, 19.0, 34.1, 176.7 (C=O); **4e**: 13.6, 18.4, 36.3, 173.4 (C=O); **4f**: 13.5, 22.2, 26.9, 34.2, 173.5 (C=O); **4g**: 26.9, 38.8, 178.1 (C=O).

The compounds **3c-3e**, which are analogs of HT-2 [**3a**] and correspond to compounds **4c**, **4d**, and **4e**, were also synthesized. Although these compounds are potential metabolites of *F. sporotrichioides* a search of the gc/ms spectra of crude fungal extracts of liquid cultures failed to detect them. This could be attributed to the predominant formation of acetylated products such at T-2 and its analogs in liquid culture.

EXPERIMENTAL

GENERAL TECHNIQUES.—^{1}H-nmr spectra were recorded at 250 MHz on a Bruker WM250 spectrometer. Spectra were acquired with 16 K data points, a 2200 Hz spectral window, 60° pulses, and an 8s repetition rate. Chemical shifts are referenced to residual $CHCl_3$ at 7.24 ppm and reported (ppm) relative to TMS. All ^{1}H-nmr data are shown in Table 1. Mass spectra were obtained on either a Finnigan MAT 312 mass spectrometer or a Finnigan MAT 4500 gc/ms system. Accurate mass measurements were determined by peak matching with an ion in the spectrum of perfluorokerosene.

PREPARATION OF DIACYL-NEOSOLANIOLS [**2**].—A solution of neosolaniol [**1**] (20 mg, 52

μmol), pyridine (34 μl, 8 eq.), and the appropriate acid chloride (10 eq.) in CH_2Cl_2 (2 ml) was stirred overnight under N_2 at room temperature. The solution was then washed with saturated aqueous $NaHCO_3$ followed by brine, dried over Na_2SO_4, and concentrated. The product was chromatographed on Si gel (3 g) with EtOAc-hexane (1:1 or 1:3, depending on the size of the acyl groups). Yields were quantitative.

ACID HYDROLYSIS TO **4**.—Compounds **2** or **5** were treated in the same manner. For instance, **2c** (13 mg, 26 μmol) was stirred overnight at room temperature in a 100:1 solution (1 ml) of MeOH and conc. HCl. The solution was then neutralized with NaOMe (8 mg) and concentrated. The residue was partitioned between H_2O and EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, concentrated, and chromatographed on Si gel (3 g) with EtOAc-hexane (1:1) followed by EtOAc. Some starting material was recovered (4.5 mg) followed by the desired product **4c** (4.5 mg) and the further deacetylated **3c** (3 mg).

Ms data for compounds **4**: **4b** calcd for $C_{21}H_{28}O_9$: 424.1733, found: 424.1749; **4c** calcd for $C_{22}H_{30}O_9$: 438.1889, found: 438.1926; **4d** calcd for $C_{23}H_{32}O_9$: 452.2046, found: 452.2064; **4e** calcd for $C_{23}H_{32}O_9$: 452.2046, found: 452.2051; **4f** calcd for $C_{24}H_{34}O_9$: 466.2202, found: 466.2203; **4g** calcd for $C_{24}H_{34}O_9$: 466.2202, found: 466.2220; **4h** calcd for $C_{22}H_{30}O_9$: 438.1889, found : 438.1830.

BASE HYDROLYSIS TO **3**.—Example: A solution of **2d** (19 mg) in MeOH (2 ml) and aqueous NH_4OH (2M, 2 ml) was stirred at room temperature for 4 h, concentrated to ½ volume, and extracted with EtOAc (3×2 ml). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Chromatography on Si gel (3 g) with EtOAc yielded **3d** (7.5 mg).

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