Journal of Natural Products
Vol. 50, No. 6, pp. 1108-1112, Nov-Dec 1987

HYPOCHLORITE-PROMOTED TRANSFORMATIONS OF TRICHOTHECENES, 3. DEOXYNIVALENOL[1]

ELIZABETH P. BURROWS*

U.S. Army Biomedical Research & Development Laboratory, Fort Detrick, Frederick, Maryland 21701-5010

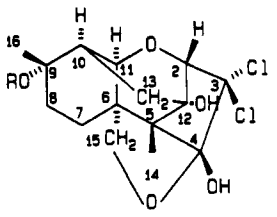
and LINDA L. SZAFRANIEC

Chemical Research, Development & Engineering Center, Aberdeen Proving Ground, Maryland 21010-5423

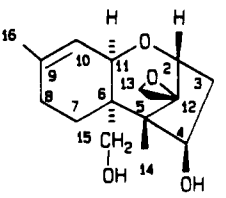
ABSTRACT.—Treatment of deoxynivalenol [**3**] in MeOH with hypochlorite bleach containing added NaOH gave rise to a single major product, the 9α,10α,12β,13β-diepoxy-8,15-hemiketal **4**. Thus, the reaction followed a very different course from that observed for verrucarol [**2**], where rearrangement involving opening of the starting 12,13-epoxide and a haloform-like oxidation took place.

Naturally occurring trichothecenes have attracted widespread attention due to their biological and physiological properties, most notably toxicity and antileukemic activity, in man and animals (1-4). Our previous investigations of chemical transformations of trichothecenes (5,6) were prompted by an observation that solutions resulting from treatment of T-2 toxin with hypochlorite bleach containing added NaOH no longer exhibited the characteristic properties of T-2 in a variety of biological assays (7). Thus, alkaline hypochlorite was recommended as a decontamination reagent for T-2 and for other trichothecenes, but the nature of the possible transformation products was not addressed. Our first product study (5) reported the isolation of two unusual pentacyclic dichlorohemiketals [**1a**, **1b**] formed in nearly quantitative yield from verrucarol [**2**], which we had chosen as a similar but simpler Type A (8) prototype for T-2. The overall reaction evidently involved several different processes: attack of solvent at C-9 resulting in opening of the epoxide and formation of the C-10, C-13 bond (9), preferential oxidation at C-4 (10) followed by α-chlorination in the manner of a haloform reaction, and cyclization to the hemiketal. These transformation products were stable to the conditions of their formation at room temperature, but, on heating with alkali, the major product **1a** underwent cleavage with rearrangement to a mixture of three C_{14} products (6).

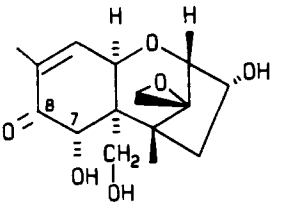
Deoxynivalenol [**3**] (DON, vomitoxin), recognized as perhaps the single most economically significant trichothecene due to its frequent occurrence in infected corn and cereal grains and its subsequent deleterious effects on livestock, has recently been extensively investigated chemically (11-15) and biologically (16,17). The presence of the C-8 carbonyl function was found to promote facile rearrangement of **3** on mild



1a R=H
1b R=Me



2



3

[1]Portions of this material were presented at the 193rd American Chemical Society National Meeting, Denver, CO, April 1987; paper ORGN 182.

treatment with alkali to a C_{15} lactone (13,14) and a mixture of three C_{14} products (14). In this paper we have extended our investigation of hypochlorite-promoted transformations to **3** and report that the reaction follows a very different course from that observed for **2** (5).

RESULTS AND DISCUSSION

In contrast to **2**, which reacted slowly and required at least 20 h for conversion to **1a**, **1b**, only 2 h were required for complete consumption of the starting **3** in the presence of hypochlorite. Again in contrast to **2**, the products obtained from **3** appeared unstable to the reaction conditions, and longer standing gave lower yields of more complex mixtures. Unlike **1a**, **1b**, these products required derivatization for characterization by gc/ms, and the predominant product, $C_{15}H_{20}O_7$, was observed as a mixture of trimethylsilyl and tetratrimethylsilyl ethers. Attempted purification by column chromatography on Si gel resulted in complex mixtures of secondary transformation products. A pure sample of the predominant product was obtained by virtue of its relative insolubility and facile crystallization from Me_2CO.

The ^{13}C- and ^1H-nmr spectra observed for the predominant product (in MeOH) are summarized in Table 1. Carbon multiplicities were determined from an APT experiment (18) and a DEPT experiment (19); proton-proton coupling information was obtained from a two-dimensional (2D) homonuclear chemical shift correlation experiment (COSY) (20); and assignments of carbons with attached protons were facilitated by a 2D heteronuclear chemical shift correlation experiment (21).

TABLE 1. ^{13}C- and ^1H-nmr Spectra of Major Transformation Product **4**

Carbon No.	^{13}C Chemical Shift (ppm)[a,b] (Multiplicity)	^1H Chemical Shift (ppm)[c,d] (Multiplicity)	J, Hz[e]
14	14.8 (q)	1.08 (s)	—
16	17.3 (q)	1.35 (s)[f]	—
5	44.3 (s)	—	—
4	45.5 (t)	1.66 (dd)	4.8, 14.4
		1.92 (dd)	10.9, 14.4
13	47.7 (t)	3.00 (d)	4.4
		3.12 (d)	4.4
6	53.9 (s)	—	—
10	58.7 (d)[g]	2.88 (s)[h]	—
12	65.5 (s)	—	—
9	67.2 (s)	—	—
15	69.0 (t)	3.39 (d)	8.2
		4.06 (d)	8.2
3	69.9 (d)	4.36 (m)	—
11	75.9 (d)	4.48 (s)	—
7	77.0 (d)	3.73 (s)	—
2	82.7 (d)	3.51 (d)	4.4
8	105.2 (s)	—	—

[a]7.6 mg in CD_3OD, sweep width 12 KHz, pulse width 4 μsec (~43°), repetition rate 3.75 sec.

[b]±0.1 ppm.

[c]Sweep width 2,600 Hz, pulse width 13.3 μsec (~65°), repetition rate 6.5 sec.

[d]±0.01 ppm.

[e]±0.2 Hz.

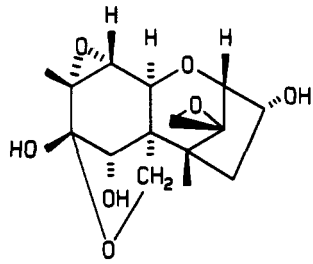
[f]Observed at 1.88 for **3**.

[g]Observed at 138.6 for **3**.

[h]Observed at 6.61 for **3**.

Comparison of these data with the published ^{13}C and ^{1}H assignments for **3** (22) revealed some significant differences. Notably, the absence of ^{13}C resonances downfield of 105.2 ppm indicated transformation of both the C-8 carbonyl group and the C-9, C-10 double bond. Significantly, however, the presence of the two ^{1}H doublets at δ 3.00 and 3.12 attached to C-13 demonstrated an intact 12,13-epoxide. The upfield chemical shift changes observed for C-10 and its attached proton (from δ 138.6 and δ 6.61 in **3** to δ 58.7 and δ 2.88, respectively, in the product) as well as for the C-16 protons (from δ 1.88 in **3** to δ 1.35 in the product) were consistent with the presence of a 9,10-epoxide. In the one-dimensional (1D) ^{1}H spectrum coupling between H-10 and H-11 was too small to measure, and the resonances at δ 2.88 and δ 4.48 were observed as singlets. The COSY experiment, however, showed a small (<1 Hz) but reproducible coupling between H-10 and H-11, in addition to the obvious geminal proton couplings at positions 13 and 15, and the H-3, H-4, H-4′ couplings. Thus, the 9α, 10α configuration followed.

The new high frequency resonance at δ 105.2 and the substantial changes in chemical shifts and coupling constants observed for the C-15 protons (from δ 3.73 and δ 3.89, J=11.6 in **3** to δ 3.39 and δ 4.06, J=8.2 in the product) were attributed to the presence of a C-8, C-15 hemiketal. Thus, the predominant product can be assigned the unique stereostructure **4**.



4

In addition to confirming the assignment of carbons with attached protons, the heteronuclear chemical shift correlation experiment revealed the resonance at δ 47.7 due to C-13 which in the 1D ^{13}C spectrum was obscured by the MeOH solvent. Assignments of the two quaternary carbons bound to oxygen, C-9 and C-12, were made on the basis of a selective decoupling experiment in which irradiation of the C-16 methyl protons resulted in sharpening of the resonance at δ 67.2, substantiating its assignment to C-9.

While epoxidation of α,β-unsaturated ketones by hypochlorite is not exceptional (23), this epoxidation of **3** is noteworthy for several reasons. 9β,10β-Epoxides of trichothecenes are the norm, as molecular models predict. They have been isolated from plant sources (24,25), and, in one recent case, from a fungal source (26).[2] They are the major products of perbenzoic or *m*-chloroperbenzoic acid oxidation of naturally occurring Type A (9,27) and macrocyclic (28-31) trichothecenes. In three cases, those of verrucarin A (29), verrucarin J (31), and roridin H (31), 9α,10α-diepoxides were isolated as minor products. For the latter two cases, $J_{10,11}$ values of 2 Hz were reported, while values for the 9β,10β compounds generally fell within the expected 5-6 Hz range.

[2]It is now generally believed that plants acquire trichothecenes from a fungal source; for a discussion see B.B. Jarvis, S.N. Çömezoğlu, M.M. Rao, N.B. Pena, F.E. Boettner, T.M. Williams, G. Forsyth, and B. Epling, *J. Org. Chem.*, **52**, 45 (1987).

In addition to the unusual 9α,10α configuration, diepoxide **4** is unique in two respects. To our knowledge, it represents the first report of 9,10 epoxidation of a Type B (8) trichothecene, and to our knowledge 8,15 hemiketal formation is also without precedent.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—^{13}C- and ^{1}H-nmr and low resolution mass spectral instrumentation and parameters are given in Burrows and Szafraniec (5). High resolution eims were determined with a VG Model 7070-EQHF instrument. Samples were treated 2 h at room temperature with $CHCl_3$-Tri-Sil TBT (1:1) (Pierce Chemical Co.) for derivatization prior to gc/ms analysis.

HYPOCHLORITE TREATMENT OF DEOXYNIVALENOL.—To a stirred, ice-cooled solution of **3** (50 mg, 0.17 mmol) (Research Foods, Ltd., Downsview, Ontario, Canada) in MeOH (5 ml) was added a 10% solution of NaOCl (50 ml, Robinson Chemical Co., Cambridge, MD) in which 1 g of NaOH had been freshly dissolved. The mixture was stirred 2 h at room temperature, then cooled during neutralization with 6N HCl (4 ml), saturated with NaCl, and extracted with 3 portions of EtOAc (combined volume 120-150 ml). Evaporation of the dried ($MgSO_4$) extracts yielded crude **4** (28.5 mg, 54%) as a white solid[3] that was purified by recrystallization from Me_2CO. Characteristics of **4**: dec. without melting 165-167°; hreims, calcd for $C_{15}H_{20}O_7$, *m/z* 312.1209, found 312.1231.

ACKNOWLEDGMENTS

We thank Dr. Harry Hines for the exact mass determination.

LITERATURE CITED

1. "Trichothecenes—Chemical, Biological and Toxicological Aspects." Ed. by Y. Ueno, Elsevier Science, Amsterdam, 1983.
2. B.B. Jarvis and E.P. Mazzola, *Acc. Chem. Res.*, **15**, 388 (1982).
3. "Mycotoxins in Human and Animal Health." Ed. by J. Rodricks, C.W. Hesseltine, and M.A. Melman, Pathotox, Park Forest South, IL, 1977, pp. 187-340.
4. C.J. Mirocha, S.V. Pathre, and C.M. Christensen, in: "Mycotoxic Fungi, Mycotoxins, and Mycotoxicoses." Ed. by T.D. Wyllie and L.G. Morehouse, Marcel Dekker, New York, 1977, vol. 1, pp. 393-409.
5. E.P. Burrows and L.L. Szafraniec, *J. Org. Chem.*, **51**, 1494 (1986).
6. E.P. Burrows and L.L. Szafraniec, *J. Org. Chem.*, **51**, 4706 (1986).
7. R.W. Wannemacher, D.L. Bunner, R.E. Dinterman, J.G. Pace, W.L. Thompson, C.F. Matson, P. Fontello, and R.W. Kuehne, "Methods for Safe Handling and Decontamination of T-2 Mycotoxin." Proceedings of 25th Biological Safety Conference, Cambridge, MA, November 1982.
8. Y. Ueno, N. Sato, K. Ishii, K. Sakai, H. Tsunoda, and M. Enamoto, *Appl. Microbiol.*, **25**, 699 (1973).
9. H.P. Sigg, R. Mauli, E. Flury, and D. Hauser, *Helv. Chim. Acta*, **48**, 962 (1965).
10. B. Muller and Ch. Tamm, *Helv. Chim. Acta*, **58**, 541 (1975).
11. R.R. King, R. Greenhalgh, and B.A. Blackwell, *J. Agric. Food Chem.*, **32**, 72 (1984).
12. R. Greenhalgh, J. Gilbert, R.R. King, B.A. Blackwell, J.R. Startin, and M.J. Shepherd, *J. Agric. Food Chem.*, **32**, 1416 (1984).
13. J.F. Grove, *J. Chem. Soc., Perkin Trans. 1*, 1731 (1985).
14. J.C. Young, B.A. Blackwell, and J.W. ApSimon, *Tetrahedron Lett.*, **27**, 1019 (1986).
15. J.C. Young, *J. Agric. Food Chem.*, **34**, 919 (1986).
16. R.R. King, R.E. McQueen, D. Levesque, and R. Greenhalgh, *J. Agric. Food Chem.*, **32**, 1181 (1984).
17. L.M. Cote, J. Nicoletti, S.P. Swanson, and W.B. Buck, *J. Agric. Food Chem.*, **34**, 458 (1986).
18. S.L. Patt and J.N. Shoolery, *J. Magn. Reson.*, **46**, 535 (1982).
19. D.M. Doddrell, D.T. Pegg, and M.R. Bendall, *J. Magn. Reson.*, **48**, 323 (1982).
20. A. Bax, R. Freeman, and G.A. Morris, *J. Magn. Reson.*, **42**, 164 (1981).
21. A. Bax and G.A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).
22. B.A. Blackwell, R. Greenhalgh, and A.D. Bain, *J. Agric. Food Chem.*, **32**, 1078 (1984).
23. S.K. Chakrabartty, in: "Oxidation in Organic Chemistry, Part C." Ed. by W.S. Trahanovski, Academic Press, New York, 1978, p. 363.

[3]In addition to **4**, small amounts of unstable monochlorinated products were present.

24. S.M. Kupchan, B.B. Jarvis, R.G. Dailey, Jr., W. Bright, R.F. Bryan, and Y. Shizuri, *J. Am. Chem. Soc.*, **98**, 7092 (1976).
25. S.M. Kupchan, D.R. Streelman, B.B. Jarvis, R.G. Dailey, Jr., and A.T. Sneden, *J. Org. Chem.*, **42**, 4221 (1977).
26. B.B. Jarvis and C.S. Yatawara, *J. Org. Chem.*, **51**, 2906 (1986).
27. T. Kaneko, H. Schmitz, J.M. Essery, W. Rose, H.G. Howell, T.A. O'Herron, S. Nachfolger, J. Huftalen, W.T. Bradner, R.A. Partyka, T.W. Doyle, J. Davies, and E. Cundliffe, *J. Med. Chem.*, **25**, 579 (1982).
28. W. Zurcher, J. Gutzwiller, and Ch. Tamm, *Helv. Chim. Acta*, **48**, 840 (1965).
29. R. Achini and Ch. Tamm, *Helv. Chim. Acta*, **51**, 1712 (1968).
30. B.B. Jarvis, G.P. Stahly, G. Pavanasasivam, and E.P. Mazzola, *J. Med. Chem.*, **23**, 1054 (1980).
31. B.B. Jarvis, J.O. Midiwo, and E.P. Mazzola, *J. Med. Chem.*, **27**, 239 (1984).

Received 26 June 1987