diminished activity. The dihydro derivative 4, which still has the spiro epoxy group, showed about half the activity of the parent tetraol 3. The most striking results, however, were obtained with compounds 5–7 in which the epoxide had been opened. These compounds were consumed at the same rate as the control solution. Even at 100 mg/L, the mixture of epimeric hydrates 6 failed to elicit a refusal response. These results generally parallel those obtained in cytotoxicity tests of similar derivatives from diacetoxyscirpenol (Grove and Mortimer, 1969). In addition, compound 5 has been shown to lack both topical and intraperitoneal toxicity to rats (Bamburg, 1972). Therefore, in terms of feed refusal and perhaps other toxicological properties as well, thermal hydration of 8-hydroxyepoxytrichothecenes offers an approach to decontamination of materials containing these toxins. T-2 toxin, which has a bulky ester substituent at position 8, requires a preliminary mild hydrolysis step to remove this group prior to hydration. The hydration approach may have potential applicability toward a detoxification process, because no hazardous or difficult to remove reagents need to be added to destroy the spiro epoxide ring.

Registry No. 1, 21259-20-1; 2, 21259-21-2; 3, 34114-99-3; 4, 89121-46-0; 5, 89121-47-1; 6, 89121-48-2; 6 tetraacetate, 89121-53-9; 7, 89121-49-3; 8, 89121-50-6; 9, 89121-51-7; 10, 89121-52-8.

LITERATURE CITED

Bamburg, J. R. *Clin. Toxicol.* **1972**, *5*, 495.
Burmeister, H. R. *Appl. Microbiol.* **1971**, *21*, 739.
Burmeister, H. R.; Vesonder, R. F.; Kwolek, W. F. *Appl. Environ. Microbiol.* **1980**, *39*, 957.
Doyle, T. W.; Bradner, W. T. In "Anticancer Agents Based on Natural Product Models"; Cassady, J. M.; Duros, J. D., Eds.; Academic Press: New York, 1980; pp 43–72.
Ellison, R. A.; Kotsonis, F. N. *J. Org. Chem.* **1976**, *41*, 576.
Grove, J. F.; Mortimer, P. H. *Biochem. Pharmacol.* **1969**, *18*, 1473.
Gutzwiller, J.; Mauli, R.; Sigg, H. P.; Tamm, Ch. *Helv. Chim. Acta* **1964**, *47*, 2234.
Kotsonis, F. N.; Ellison, R. A.; Smalley, E. B. *Appl. Microbiol.* **1975**, *30*, 493.
Pathre, S. V.; Mirocha, C. J. In "Mycotoxins in Human and Animal Health"; Rodricks, J. V.; Hesseltine, C. W.; Mehlman, M. A., Eds.; Pathotox Publishers, Inc.: Park Forest South, IL, 1977; pp 229–253.
Sato, N.; Ueno, Y. In "Mycotoxins in Human and Animal Health"; Rodricks, J. V.; Hesseltine, C. W.; Mehlman, M. A., Eds.; Pathotox Publishers, Inc.: Park Forest South, IL, 1977; pp 295–307.
Sigg, H. P.; Mauli, R.; Flury, E.; Hauser, D. *Helv. Chim. Acta* **1965**, *48*, 962.
Vesonder, R. F.; Ciegler, A.; Burmeister, H. R.; Jensen, A. H. *Appl. Environ. Microbiol.* **1979**, *38*, 344.

Received for review September 12, 1983. Revised manuscript received January 17, 1984. Accepted January 24, 1984. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Mass Spectrometry of Cytochalasin H and Its Acetyl and Deacetyl Analogues

Donald F. Magin,* Richard H. Cox, W. Noel Einolf, and Horace G. Cutler

A study was made of the electron ionization (EI) and chemical ionization (CI) (reagent gases: methane and ammonia) mass spectra of cytochalasin H and some of its analogues. The purpose of this work was to characterize these compounds through a study of their mass spectra under EI and CI conditions. The mass spectra are shown to contain all of the information necessary to determine the degree of acetylation as well as the presence of an acyl group on the nitrogen atom. Losses can be followed, accounting for all hydroxyl or acetyl groups on carbons 7, 18, and/or 21 and for an acyl group on the nitrogen atom.

The cytochalasins, metabolites generated by a variety of molds found growing on cereal crops, produce unusual biological effects including inhibition of cell movement, inhibition of cytoplasmic cleavage, and nuclear extrusion of cultured cells. Some cytochalasins have been reported to be acutely toxic to experimental animals (Natori, 1977). In addition, several have been shown to exhibit plant growth inhibitory activity in wheat coleoptiles (Cole et al., 1981; Cutler et al., 1980). During the course of investigation of the plant growth regulating effects of acetate derivatives of cytochalasin H (Beno et al., 1977; Cole et al., 1981; Patwardhan et al., 1974; Wells et al., 1976), it became apparent that the electron impact mass spectra of some of the acetate derivatives did not yield abundant molecular ions with large enough intensity to be useful for characterization purposes (Cox et al., 1983). In order to characterize these cytochalasins more fully, the mass spectral properties of cytochalasin H and its acetyl and deacetyl analogues (Figure 1, 1–5) were investigated further. We report here the results of our study of their electron ionization (EI) and chemical ionization (CI) mass spectra. The mass spectral examinations of the cytochalasin H series of compounds were facilitated by using the technique of desorption chemical ionization (DCI).

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EXPERIMENTAL SECTION

The isolation, purification, and structure elucidation of the cytochalasin H series of compounds has been described previously by Cox et al. (1983).

Chemical ionization and electron ionization mass spectra were obtained by using a Varian MAT 112S mass spectrometer coupled with a Varian MAT SS-200 data system. [The mass spectra under EI and CI conditions are available

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Table I. Methane CI Data [*m/z* (Relative Intensity)][a]

ion (+)	1[b]	2	3	4	5	apparent losses from M + 1 ion
M + 1	452 (2)	494 (18)	536 (12)	578 (12)	620 (24)	
M	451 (1)	493 (6)	535 (1)	577 (5)	619 (1)	H
M − 1	450 (3)	492 (12)	534 (6)	576 (6)		2H
M − 16		477 (25)	519 (14)	561 (20)		OH
M − 17	434 (7)	476 (69)	518 (36)	560 (54)		H_2O
M − 33	418 (1)	460 (1)				2OH
M − 34	417 (2)	459 (4)				OH + H_2O
M − 35	416 (6)	458 (10)				$2H_2O$
M − 41				536 (4)	578 (5)	C_2H_2O
M − 53	398 (1)					$3H_2O$
M − 58		435 (31)	477 (28)	519 (40)	561 (35)	OAc
M − 59		434 (100)	476 (100)	518 (100)	560 (100)	HOAc
M − 75		418 (5)	460 (2)	502 (3)		OAc + OH
M − 76		417 (16)	459 (9)	501 (11)		OAc + H_2O or HOAc + OH
M − 77		416 (53)	458 (23)	500 (29)		HOAc + H_2O
M − 93		400 (1)				HOAc + 2OH or OAc + OH + H_2O
M − 94		399 (4)				HOAc + H_2O + OH or $2H_2O$ + OAc
M − 95		398 (10)				HOAc + $2H_2O$
M − 100				477 (8)	519 (8)	OAc + C_2H_2O
M − 101				476 (20)	518 (23)	HOAc + C_2H_2O
M − 117			418 (2)	460 (3)	502 (6)	2OAc
M − 118			417 (11)	459 (15)	501 (21)	OAc + HOAc
M − 119			416 (42)	458 (47)	500 (60)	2HOAc
M − 135				442 (2)		2OAc + H_2O or HOAc + OH + H_2O
M − 136			399 (5)	441 (7)		HOAc + OAc + H_2O or 2HOAc + OH
M − 137			398 (12)	440 (21)		2HOAc + H_2O
M − 160				417 (2)	459 (9)	HOAc + OAc + C_2H_2O
M − 161				416 (7)	458 (25)	2HOAc + C_2H_2O
M − 177					442 (3)	HOAc + 2OAc
M − 178[c]				399 (2)	441 (11)	2HOAc + OAc or HOAc + OAc + H_2O + C_2H_2O
M − 179[d]				398 (5)	440 (36)	3HOAc or 2HOAc + H_2O + C_2H_2O
M − 221					398 (2)	3HOAc + C_2H_2O

[a] Compounds 1–5 from Figure 1. [b] Base peak for 1 was at *m/z* 85. [c] HOAc + OAc + H_2O + C_2H_2O from 4; 2HOAc + OAc from 5. [d] 2HOAc + H_2O + C_2H_2O from 4; 3HOAc from 5.

Table II.[a] Ammonia CI Data [*m/z* (Relative Intensity)][b]

ion (+)	1[c]	2[d]	3	4	5	apparent losses from M + 18 ion
M + 18	469 (10)	511 (13)	553 (100)	595 (100)	637 (100)	
M	451 (2)	493 (13)	535 (11)	577 (12)		H_2O
M − 18		475 (1)				$2H_2O$
M − 24				553 (5)	595 (12)	C_2H_2O
M − 42[e]		451 (8)	493 (10)	535 (8)	577 (44)	HOAc or C_2H_2O + H_2O
M − 60		433 (3)				HOAc + H_2O
M − 78		415 (2)				HOAc + $2H_2O$
M − 84					535 (2)	HOAc + C_2H_2O
M − 102			433 (7)	475 (2)	517 (2)	2HOAc
M − 120				457 (2)		2HOAc + H_2O
M − 164					457 (1)	3HOAc

[a] This table identifies those mass peaks in the CI (NH_3) experiments that may be attributed to losses of neutral molecules from the $(M + NH_4)^+$ adduct ions. Other ions were seen that correspond to losses from protonated molecular ions. These are identified in Table I, CI (CH_4) data. See the text for further explanation. [b] Compounds 1–5 from Figure 1. [c] Base peak for 1 was at *m/z* 101. [d] Base peak for 2 was at *m/z* 476 (loss of H_2O from M + 1 ion). [e] C_2H_2O + H_2O from 4 only.

as supplementary material (see paragraph at end of paper regarding supplementary material).] Samples for electron ionization (EI) MS were introduced through the direct probe inlet at a probe temperature and source temperature of 200–250 °C. Samples for desorption chemical ionization (CI) MS were introduced on the tip of an unheated Vespel DCI probe of our own design (Einolf, 1983), based on work reported by Hunt et al. (1977) Cotter (1979), and Hansen and Munson (1980), into a source heated at 200 °C. Reagent gases used for CIMS were methane, isobutane, and ammonia. Source pressures under CI conditions were 0.5–0.7 torr, calculated by using the method of Hancock et al. (1979). Because of the similarity between spectra obtained with isobutane reagent gas to those obtained by using methane, the isobutane results are not reported here.

The spectra under CI (C_4H_{10}) conditions did show small (<10% relative intensity) ions at M + 29 and M + 41, but the patterns of the rest of the spectra were similar to those of the CI (CH_4) spectra.

RESULTS AND DISCUSSION

Chemical Ionization Mass Spectrometry (CIMS). *Losses from the Protonated Molecular Ion.* The methane (CH_4) and the ammonia (NH_3) CI results will be discussed together, since the fragmentation from the M + 1 ion generated under CI (NH_3) conditions is very similar to the results from the CI (CH_4) experiments. Fragmentation patterns generated from the ammonium adduct ion $(M + 18)^+$ in CI (NH_3) will be discussed in a separate section.

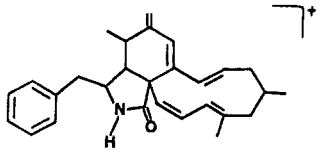
The CIMS data for the cytochalasin H series of compounds (1–5) analyzed with methane reagent gas are summarized in Table I. The fragmentation patterns for all compounds have certain common factors. First, fragmentations (either stepwise or concerted) can be followed

Table III. Prominent Ions in EI Spectra [m/z (Relative Intensity)][a]

ion (+)	1	2[b]	3	4	5	apparent losses from M^+ ion[c]
M^+	451 (1)	493 (6)	535 (4)	577 (1)	619 (1)	
M − 1	450 (1)					H
M − 2	449 (3)					2H
M − 17	434 (9)	476 (1)	518 (2)	560 (1)		OH
M − 18	433 (31)	475 (1)				H_2O
M − 19	432 (3)					H_2O + H
M − 20	431 (4)					H_2O + 2H
M − 34	417 (1)					2OH
M − 35	416 (4)					H_2O + OH
M − 36	415 (7)					$2H_2O$
M − 42					577 (1)	C_2H_2O
M − 51	400 (4)					3OH
M − 53	398 (1)					$2H_2O$ + OH
M − 54	397 (2)					$3H_2O$
M − 59		434 (7)	476 (4)	518 (1)	560 (6)	OAc
M − 60		433 (6)	475 (1)	517 (2)	559 (15)	HOAc
M − 61		432 (2)				HOAc + H
M − 77		416 (11)	458 (1)			HOAc + OH or OAc + H_2O
M − 78		415 (13)	457 (1)			HOAc + H_2O
M − 91	360 (3)	402 (15)	444 (6)	486 (1)	528 (1)	$PhCH_2$
M − 92	359 (9)					$PhCH_2$ + H
M − 93	358 (32)					$PhCH_2$ + 2H
M − 108	343 (6)	385 (1)				$PhCH_2$ + OH
M − 109	342 (21)	384 (4)				$PhCH_2$ + H_2O
M − 118			417 (2)	459 (1)	501 (1)	2OAc
M − 119			416 (7)	458 (3)	500 (1)	HOAc + OAc
M − 120			415 (9)	457 (6)	499 (1)	2HOAc
M − 125	326 (2)					$PhCH_2$ + 2OH
M − 126	325 (3)					$PhCH_2$ + H_2O + OH
M − 127	324 (14)					$PhCH_2$ + $2H_2O$
M − 137			398 (6)	440 (2)		2HOAc + OH or HOAc + H_2O + OAc
M − 138			397 (6)	439 (3)		2HOAc + H_2O
M − 142	309 (1)					$PhCH_2$ + 3OH
M − 143	308 (2)					$PhCH_2$ + H_2O + 2OH
M − 144	307 (2)					$PhCH_2$ + $2H_2O$ + OH
M − 145	306 (5)					$PhCH_2$ + $3H_2O$
M − 150		343 (1)				$PhCH_2$ + OAc
M − 151		342 (7)	384 (2)	426 (1)	468 (1)	$PhCH_2HOAc$
M − 160				417 (1)	459 (2)	C_2H_2O + 2OAc
M − 161				416 (2)	458 (5)	OAc + HOAc + C_2H_2O
M − 162				415 (2)	457 (1)	2HOAc + C_2H_2O
M − 168		325 (5)	367 (1)			$PhCH_2$ + HOAc + OH or $PhCH_2$ + OAc + H_2O
M − 169		324 (22)	366 (2)	408 (1)		$PhCH_2HOAc$ + H_2O
M − 179				398 (3)	440 (13)	2HOAc + OAc
M − 180				397 (5)	439 (3)	3HOAc
M − 187		306 (5)				$PhCH_2$ + $2H_2O$ + OAc
M − 210			325 (3)	367 (1)		$PhCH_2$ + HOAc + OAc
M − 211			324 (13)	366 (3)	408 (1)	$PhCH_2$ + 2HOAc
M − 220					399 (3)	HOAc + 2OAc + C_2H_2O
M − 221					398 (8)	2HOAc + OAc + C_2H_2O
M − 222					397 (3)	3HOAc + C_2H_2O
M − 228			307 (2)			2HOAc + $PhCH_2$ + OH or OAc + HOAc + $PhCH_2$ + H_2O
M − 229			306 (8)			2HOAc + $PhCH_2$ + H_2O
M − 252				325 (2)	367 (1)	HOAc + $PhCH_2$ + OAc + C_2H_2O
M − 253				324 (10)	366 (5)	2HOAc + $PhCH_2$ + C_2H_2O
M − 270				307 (2)	349 (1)	2HOAc + $PhCH_2$ + OAc
M − 271				306 (7)	348 (2)	3HOAc + $PhCH_2$
M − 310					309 (1)	3OAc + $PhCH_2$ + C_2H_2O
M − 311					308 (1)	2OAc + HOAc + $PhCH_2$ + C_2H_2O
M − 312					307 (2)	OAc + 2HOAc + $PhCH_2$ + C_2H_2O
M − 313					306 (8)	3HOAc + $PhCH_2$ + C_2H_2O
91	91 (100)	91 (75)	91 (53)	91 (29)	91 (26)	this is the $PhCH_2$ ion

[a] Compounds 1-5 from Figure 1. [b] Base peak for 2-5 was at m/z 43. [c] Ph = C_6H_5 (phenyl).

for all of the cytochalasins to an ion at m/z 398. The structure of this ion is postulated to be **6**, although other



6

structures are possible. In addition, the most intense peak in each spectrum in the mass range 398 to $(M + 1)^+$ is an ion resulting from the loss of a single neutral species, either water or acetic acid, from the M + 1 ion. The water arises from an hydroxyl group at carbons 7, 18, or 21, and the acetic acid from an acetate group from one of the same carbons. For the di- and triacetylated cytochalasins (4 and 5), another loss from the $(M + 1)^+$ ion is noted, that of the elements of ketene (C_2H_2O, 42 u). For simplicity in this paper, the loss of 42 u will be referred to as a loss of ketene,

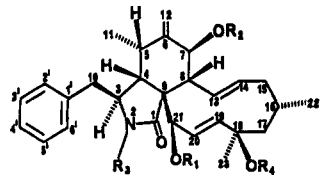


Figure 1. Structures of cytochalasin H compounds. 1, deacetylcytochalasin H: $R_1 = R_2 = R_3 = R_4 = H$. 2, cytochalasin H: $R_1 = COCH_3$; $R_2 = R_3 = R_4 = H$. 3, acetylcytochalasin H: $R_1 = R_2 = R_3 = COCH_3$; $R_4 = H$. 4, diacetylcytochalasin H: $R_1 = R_2 = R_3 = COCH_3$; $R_4 = H$. 5, triacetylcytochalasin H: $R_1 = R_2 = R_3 = R_4 = COCH_3$.

although no definitive work was done to prove that the fragment lost is actually a neutral molecule of ketene. Empirically, it appears that ketene arises from the acyl group on the nitrogen in the di- and triacetylated cytochalasins. These two compounds are the only molecules of the series to show peaks corresponding to $(M + 1 - C_2H_2O)^+$ and are the only ones to have an acyl group on the nitrogen atom. Careful examination of the data in Table I will reveal that ions can be observed representing loss of each hydroxyl, acetyl, and acyl group and of all combinations thereof.

In addition to these losses of neutral molecules, most of the ions in the spectra can be explained by including losses due to radicals or stepwise or simultaneous losses of two or more radicals and neutrals. For example, the significant peaks in the CI (CH_4) spectrum of cytochalasin H (2) can be seen at m/z 477, 459, 435, 418, 417, and 399 and are explainable by losses from the $(M + 1)^+$ ion of OH, OH + H_2O, OAc, OAc + OH, HOAc + OH (or OAc + H_2O), and HOAc + H_2O + OH (or OAc + $2H_2O$), respectively. Similar losses can be found to explain the major peaks in the other CI (CH_4) spectra.

Losses from the $(M + NH_4)^+$ Adduct Ion [CI(NH_3)]. Several ions in the CI(NH_3) spectra of the cytochalasins can be attributed to losses of neutral molecules from the M + NH_4 adduct ion $(M + 18)^+$. These ions are identified in Table II.

Unlike the schemes for losses from the $(M + 1)^+$ ion (Table I), fragments are not seen for the loss of every possible combination of water, acetic acid, and ketene. The assumption made is that the $(M + 18)^+$ ion is more stable compared to the $(M + 1)^+$ ion and that there is insufficient energy for fragmentation involving all of the substituents. Thus, losses are noted that are attributable to only some of the functional groups. Immediately obvious is the fact that the fragmentation schemes from the $(M + 18)^+$ ion result in different product ions for the different cytochalasins. Coupled with this is the observation that in contrast to the behavior of the $(M + H)^+$ ion in which all pathways lead to a product ion at m/z 398, all fragmentation pathways for the $(M + 18)^+$ adduct do not lead to the same product ion. For example, a loss of water from the $(M + 18)^+$ ion for acetylcytochalasin H (3) results in an ion that apparently does not fragment further, and the ions explainable by losses of one and two acetic acid molecules are generated by a different pathway.

The most likely point of attachment for the NH_4^+ reactant ion is at the nitrogen atom. Evidence for this comes from the case of the cytochalasin H (2), which exhibits an ion at m/z 415, due to losses of two molecules of water and one of acetic acid from the functional groups on carbons 7, 18, and 21. This ion is equivalent to a fragment of m/z 397 plus the ammonium reactant ion. Thus, the m/z 415 ion is analogous to the ion in the earlier M + 1 schemes (Table I) at m/z 398.

For the CI (NH_3) cases, the $(M + 1)^+$ ion could arise from either of two routes: as a loss of ammonia from the $(M + NH_4)^+$ adduct ion or as a direct result of protonation of the sample molecule by the NH_4^+ reactant ion. Since the schemes of fragmentation produced from this $(M + 1)^+$ ion are so similar to those for the CI (CH_4) case, identities of the ions were presented as losses of neutrals from a protonated molecular ion. Losses involving NH_3 or NH_3 plus other neutrals from the $(M + NH_4)^+$ adduct ion were thus purposely omitted from Table II for the sake of simplicity, since those same ions as identified in the CI (CH_4) experiments as losses from an $(MH)^+$ ion could have originated under CI (NH_3) conditions as losses from the $(M + NH_4)^+$ adduct ion of a combination of ammonia (NH_3) plus the corresponding species (see Table I).

Electron Impact Mass Spectrometry (EIMS). There is a definite analogy between the EI and the CI mass spectra. For each of the cytochalasins (1–5), losses can be traced to a peak at m/z 306 in the EI spectra. This relates to the m/z 398 ion seen in the CI spectra in the following way. If the "backbone" of the cytochalasin H series is viewed as the structure minus substituents (except for hydrogens) on the nitrogen and carbons 7, 18, and 21, then the m/z 398 ion in the CI spectra is the protonated "backbone", and the m/z 306 ion in the EI spectra is this "backbone" minus the benzyl fragment. An ion at m/z 91 (benzyl) appears in all of the EI spectra as a prominent peak. Other important peaks in the EI spectra have been identified and are listed in Table III. Prominent in the spectra are losses attributable to hydrogen atoms, water, acetic acid, ketene, and radicals such as hydroxyl, acetyl, and benzyl.

Note that beginning with the diacetylcytochalasin H (4), fragments explainable by losses involving ketene are noticeable. As mentioned earlier in the CI discussion, since these losses are seen only in cases where the nitrogen atom has an acyl substituent, the supposition is that the ketene is generated from this substituent.

CONCLUSION

The EI and CI mass spectra of the cytochalasin H series of molecules have been examined in some detail and are shown to contain all the information necessary to determine the degree of acetylation as well as the presence of an acyl group on the nitrogen atom. Losses, either by stepwise fragmentations or by some concerted combinations, can be followed, accounting for the presence of all hydroxyl or acetyl groups on carbons 7, 18, and/or 21 and the presence or absence of an acyl group on the nitrogen atom.

Registry No. 1, 53760-20-6; 2, 53760-19-3; 3, 84499-89-8; 4, 84499-90-1; 5, 84499-91-2.

Supplementary Material Available: The mass spectra of the five cytochalasin H molecules under EI and methane and ammonia CI conditions (15 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

Beno, M. A.; Cox, R. H.; Wells, J. M.; Cole, R. J.; Kirksey, J. W.; Christoph, G. G. *J. Am. Chem. Soc.* **1977**, *99*, 4123–4130.

Cole, R. J.; Wells, J. M.; Cox, R. H.; Cutler, H. G. *J. Agric. Food Chem.* **1981**, *29*, 205–206.

Cotter, R. J. *Anal. Chem.* **1979**, *51* (2), 317–318.

Cox, R. H.; Cutler, H. G.; Hurd, R. E.; Cole, R. J. *J. Agric. Food Chem.* **1983**, *31*, 405–408.

Cutler, H. G.; Crumley, F. G.; Cox, R. H.; Cole, R. J.; Darner, J. W.; Springer, J. P.; Laterell, F. M.; Thean, J. A.; Rossi, A. E. *J. Agric. Food Chem.* **1980**, *28*, 139–142.

Einolf, W. N. "The Mass Spec Handbook of Service"; Scientific Instrument Services, Inc.: Pennington, NJ, 1983; Vol. 1.

Hancock, R. A.; Walder, R.; Weigel, H. *Org. Mass Spectrom.* **1979**, *14*, 507–511.
Hansen, G.; Munson, B. *Anal. Chem.* **1980**, *52*, 245–248.
Hunt, D. F.; Shabanowitz, J.; Botz, F. K.; Brent, D. A. *Anal. Chem.* **1977**, *49*, 1160–1163.
Natori, S. In "Mycotoxins in Human and Animal Health"; Rodricks, J. W.; Hasseltine, C. W.; Mehlman, M. A., Eds.; Pathatox Publishers, Inc.: Park Forest South, IL, 1977; p 559.
Patwardhan, S. A.; Pandey, R. C.; Dev, S.; Pendse, C. S. *Phytochemistry* **1974**, *13*, 1985–1988.
Wells, J. M.; Cutler, H. G.; Cole, R. J. *Can. J. Microbiol.* **1976**, *22*, 1137–1143.

Received for review July 28, 1983. Revised manuscript received December 19, 1983. Accepted January 9, 1984.

Gas Chromatographic Method for the Determination of Solanidine and Its Application to a Study of Feed–Milk Transfer in the Cow

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An extraction method based on alkaline saponification of milk, partitioned into toluene, and subsequent gas chromatography quantitation has been developed to estimate the glycoalkaloid metabolite solanidine in bovine milk. Spiking studies indicated approximately 93% recovery of added solanidine at levels of 1.12, 0.56, and 0.28 ppm with a detection limit of 0.14 ppm. A study to determine the presence of solanidine in milk from cows consuming diets containing 0, 10, and 20% tater meal showed no detectable amounts of solanidine in any of the milk samples at 60 and 150 days of lactation.

Today processed potatoes represent 57% of the total potato production in the United States and the perentage continues to rise (Thorton and Sieczka, 1980). Considerable waste is generated during processing, and one means of utilizing it is the production of animal feed like tater meal, which is a potato waste product comprised of cull potatoes and process byproducts such as peel, screen, drum, French fry, and filter cake wastes. Studies conducted by Bushway et al. (1980b) and Bushway (1982) have shown that these waste products contain high levels of naturally occurring toxicants, potato glycoalkaloids, which at elevated levels can impart a bitter flavor to tubers and are toxic to man and animals (Sinden and Deahl, 1976; Hansen, 1925; Willimott, 1933; Jadhav and Salunkhe, 1975; Maga, 1980).

In Maine and Canada much of the potato waste is fed to dairy cattle as a feed supplement. A recent investigation by King and McQueen (1981) has demonstrated that rumen microorganisms cleave glycoalkaloids, yielding solanidine, its dihydro analogue 5β-solanidan-3β-ol, and monosaccharides. Although very little research has been performed on the toxicological and physiological properties of solanidine, there is evidence that suggests that it is similar to the glycoalkaloids (α-chaconine and α-solanine) as far as bitterness (Zitnak, 1961). The toxicological data are conflicting. Some studies indicate solanidine is more toxic than the glycoalkaloids (Nair et al., 1981; Zitnak, 1961) while others have demonstrated it is less toxic (Nishie et al., 1971, 1975). Physiological and toxicological properties of the dihydro form of solanidine are unknown.

Because of the extensive use of potato waste (which contains approximately 400 ppm of solanidine in the form of glycoalkaloids) as animal feed for dairy cows, the possible detrimental effects of solanidine, and the large consumption of milk and milk products, an investigation was conducted to develop a gas–liquid chromatographic method to quantify solanidine in whole milk and to determine if a feed ration containing potato byproducts resulted in the passage of solanidine into the milk of lactating dairy cows.

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EXPERIMENTAL SECTION

Reagents. A mixture of α-chaconine and α-solanine extracted from Katahdin potato blossoms using the procedure of Bushway et al. (1980a) was hydrolyzed following the method of Coxon et al. (1979) to obtain solanidine standard. For extractions, certified-grade solvents were used with the exception of methanol, which was purified grade (Fisher Scientific Co., Medford, MA). HPLC-grade solvents were employed for HPLC analysis (Fisher Scientific Co.). Sodium hydroxide, potassium hydroxide, and sodium sulfate were obtained from Fisher Scientific Co. Dragendorff's reagent was purchased from Sigma Chemical Co. (St. Louis, MO). The column packing, 3% OV-17 on 80–100-mesh Chromosorb W HP, was obtained from Supelco, Inc. (Bellefonte, PA).

Gas Chromatography (GC). The GC conditions for the detection of solanidine were those of King (1980) with slight modifications. Equipment included a Perkin-Elmer Signa-2 gas chromatograph with a nitrogen–phosphorus detector (Perkin-Elmer Corp., Norwalk, CT) and a Hewlett-Packard 3390 A reporting integrator (Hewlett-Packard Avondale Division, Avondale, PA).

A 1.2 m × 2 mm i.d. glass column was packed with 3% OV-17 on 80–100-mesh Chromosorb W HP. Helium (25 mL/min) was employed as a carrier gas while hydrogen (1–2 mL/min) and compressed air (600 mL/min) were used for the nitrogen–phosphorus detector. Operating temperatures were as follows: injection port, 285 °C; oven, 265 °C; detector, 290 °C; bead, 550 °C.

Thin-Layer Chromatography (TLC). Separation of solanidine was achieved on 10 × 10 cm, 200-μm thickness, Whatman high-performance thin-layer chromatographic plates (Whatman, Inc., Clifton, NJ). Plates were developed with the lower layer of a mixture of methanol–chloroform–1% ammonium hydroxide (100:100:50 v/v). Solan-

0021-8561/84/1432-0548$01.50/0