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ACKNOWLEDGMENTS AND ADDRESSES

Received May 12, 1972, from the *Drug Plant Laboratory, School of Pharmacy, University of Washington, Seattle, WA 98195*

Accepted for publication June 27, 1972.

Presented to the Pharmacognosy and Natural Products Section, APhA Academy of Pharmaceutical Sciences, Houston meeting, April 1972.

Supported in part by National Institutes of Health Research Grant GM 07515-11.

The authors are indebted to Mr. Paul Hiranaka, formerly School of Pharmacy, University of Washington, for assistance with the syntheses of some compounds.

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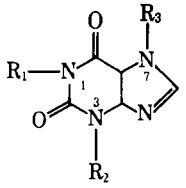
Mass Spectrometric Identification of Methylxanthines and Methyluric Acids, the Possible Metabolites of Caffeine

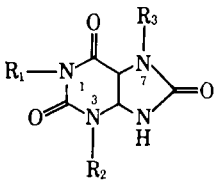
G. S. RAO*▲, K. L. KHANNA†, and H. H. CORNISH†

Abstract □ A rapid and direct mass spectrometric method for the identification of caffeine and its 15 possible metabolites formulated as tri-, di-, and monomethyl derivatives of xanthine and uric acid is described. With the exception of uric acid and its 7-methyl derivative, the molecular ion itself was found to be the base peak in the mass spectra of all of the compounds studied. Fragmentation mechanisms, along with the relative intensities of the major fragments and data on metastable ions useful in the identification of these compounds, are reported.

Keyphrases □ Caffeine metabolites, possible—mass spectrometric identification of methylxanthines and methyluric acids □ Methyluric acids—mass spectrometric identification, possible metabolites of caffeine □ Methylxanthines—mass spectrometric identification, possible metabolites of caffeine □ Mass spectroscopy—identification of methylxanthines and methyluric acids as possible metabolites of caffeine

The versatility of mass spectrometric techniques in the identification of trace quantities of drugs and drug metabolites is well recognized (1–4). As a part of studies on caffeine metabolism (5), the mass spectrometric identification of caffeine metabolites was investigated, and this paper describes the procedure for the identification of caffeine and its 15 possible metabolites containing an intact purine ring. Since *N*-dealkylation is a common metabolic reaction, the mass spectrometric behavior was studied of all possible tri-, di-, and monomethyl derivatives of xanthine and uric acid as well as the two parent compounds. Of these 16 compounds, caffeine, theobromine, theophylline, xanthine, and uric acid were previously subjected to mass spectrometric studies (6–9).





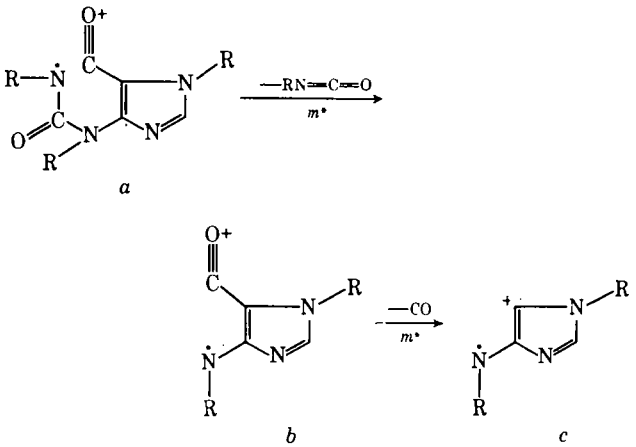
I: $R_1 = R_2 = R_3 = CH_3$
II: $R_1 = H$, $R_2 = R_3 = CH_3$
III: $R_1 = R_3 = CH_3$, $R_2 = H$
IV: $R_1 = R_2 = CH_3$, $R_3 = H$
V: $R_1 = CH_3$, $R_2 = R_3 = H$
VI: $R_1 = R_3 = H$, $R_2 = CH_3$
VII: $R_1 = R_2 = H$, $R_3 = CH_3$
VIII: $R_1 = R_2 = R_3 = H$

IX: $R_1 = R_2 = R_3 = CH_3$
X: $R_1 = R_2 = CH_3$, $R_3 = H$
XI: $R_1 = R_3 = CH_3$, $R_2 = H$
XII: $R_1 = H$, $R_2 = R_3 = CH_3$
XIII: $R_1 = CH_3$, $R_2 = R_3 = H$
XIV: $R_1 = R_3 = H$, $R_2 = CH_3$
XV: $R_1 = R_2 = H$, $R_3 = CH_3$
XVI: $R_1 = R_2 = R_3 = H$

RESULTS AND DISCUSSION

Due to the presence of heteroaromatic rings, purine derivatives generally form highly stabilized molecular ions under electron impact. All the methylxanthines and methyluric acids studied were found to exhibit intense molecular ions in their mass spectra; with the exception of uric acid and its 7-methyl derivative, the molecular ion itself was the base peak in all of the spectra. Tables I and II list the major peaks observed in the mass spectra of the 16 compounds studied. Schemes I–III describe the proposed fragmentation pathways leading to the various peaks seen in the spectra. The suggested fragmentation steps are supported by the presence of requisite metastable ions in the spectra of all the compounds examined and these are listed in Tables III and IV.

The results are in good agreement with the earlier mass spectral studies (6–9) on caffeine, theobromine, theophylline, xanthine, and uric acid derivatives (Schemes I–III). A molecule of either methylisocyanate or isocyanic acid is first expelled from the molecular radical ions (*a* and *d*), depending upon the presence or absence of the



Scheme I

Table I—Mass Spectra of Xanthine Derivatives

Number	Compound	Probe Temperature	Molecular Weight	Base Peak	Major Peaks, *m/e* (Relative Intensity) 1	2	3	4	5	6	7	8	9	10
I	Caffeine (1,3,7-Trimethylxanthine)	30°	194	194	109 (56)	55 (38)	67 (29)	82 (23)	43 (13)	137 (7)	165 (6)	136 (5)		
II	Theobromine (3,7-Dimethylxanthine)	30°	180	180	55 (64)	43 (37)	109 (34)	82 (32)	67 (30)	41 (28)	69 (22)	97 (15)	137 (13)	136 (8)
III	Paraxanthine (1,7-Dimethylxanthine)	30°	180	180	68 (82)	123 (47)	53 (24)	42 (13)	95 (12)	150 (10)	151 (9)	67 (9)	41 (8)	
IV	Theophylline (1,3-Dimethylxanthine)	30°	180	180	95 (60)	68 (44)	41 (34)	43 (22)	53 (20)	57 (19)	55 (18)	123 (15)	83 (10)	151 (9)
V	1-Methylxanthine	40°	166	166	54 (70)	109 (67)	53 (21)	81 (20)	136 (15)	137 (15)				
VI	3-Methylxanthine	40°	166	166	68 (55)	95 (38)	41 (32)	53 (31)	123 (30)					
VII	7-Methylxanthine	40°	166	166	68 (90)	123 (45)	53 (33)	42 (18)	41 (13)	95 (8)				
VIII	Xanthine	40°	152	152	54 (95)	109 (75)	53 (27)	81 (12)						

Table II—Mass Spectra of Uric Acid Derivatives

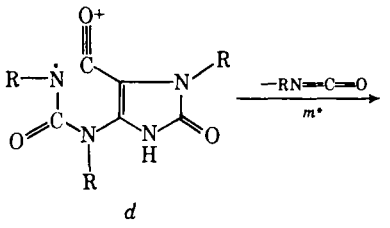
Number	Compound	Probe Temperature	Molecular Weight	Base Peak	Major Peaks, *m/e* (Relative Intensity) 1	2	3	4	5	6	7	8	9	10
IX	1,3,7-Trimethyluric acid	40°	210	210	82 (76)	67 (40)	153 (28)	125 (24)	42 (20)	55 (10)				
X	1,3-Dimethyluric acid	40°	196	196	68 (80)	139 (56)	83 (36)	53 (36)	56 (24)	111 (17)	55 (17)			
XI	1,7-Dimethyluric acid	45°	196	196	68 (70)	43 (30)	82 (28)	111 (27)	139 (25)	53 (20)	153 (16)			
XII	3,7-Dimethyluric acid	45°	196	196	82 (72)	67 (55)	153 (40)	42 (25)	125 (20)	55 (15)				
XIII	1-Methyluric acid	130°	182	182	54 (63)	68 (38)	125 (36)	139 (32)	53 (28)	97 (22)	83 (19)	111 (12)		
XIV	3-Methyluric acid	90°	182	182	68 (60)	139 (50)	83 (40)	53 (36)	54 (35)	125 (20)	111 (15)	97 (15)	41 (15)	42 (14)
XV	7-Methyluric acid	100°	182	68	182 (95)	139 (62)	53 (38)	42 (38)	69 (25)	111 (24)	41 (16)	83 (14)		
XVI	Uric acid	210°	168	54	125 (97)	168 (80)	69 (38)	97 (25)	53 (25)	43 (23)				

Table III—Metastable Ions Present in Mass Spectra of Xanthine Derivatives

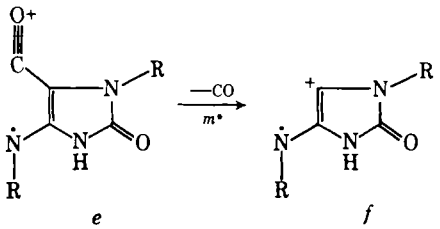
Number	Compound	Transition	Calculated *m**	Found *m**	Fragment Expelled
I	Caffeine	137 → 109	86.7	86.6	Carbon monoxide
		109 → 82	61.6	61.5	Hydrogen cyanide
II	Theobromine	180 → 137	104.2	104.5	Isocyanic acid
		137 → 109	86.7	86.6	Carbon monoxide
		109 → 82	61.6	61.5	Hydrogen cyanide
III	Paraxanthine	180 → 123	84.0	84.2	Methylisocyanate
		123 → 95	73.4	73.5	Carbon monoxide
		95 → 68	48.7	48.5	Hydrogen cyanide
IV	Theophylline	180 → 123	84.0	84.3	Methylisocyanate
		123 → 95	73.4	73.5	Carbon monoxide
		95 → 68	48.7	48.6	Hydrogen cyanide
V	1-Methylxanthine	166 → 109	71.6	71.5	Methylisocyanate
		109 → 81	60.1	60.0	Carbon monoxide
VI	3-Methylxanthine	166 → 123	91.2	91.5	Isocyanic acid
		123 → 95	73.4	73.5	Carbon monoxide
		95 → 68	48.7	48.8	Hydrogen cyanide
VII	7-Methylxanthine	166 → 123	91.2	91.2	Isocyanic acid
		123 → 95	73.4	73.4	Carbon monoxide
		95 → 68	48.7	48.7	Hydrogen cyanide
VIII	Xanthine	152 → 109	78.2	78.2	Isocyanic acid
		109 → 81	60.1	60.0	Carbon monoxide

Table IV—Metastable Ions Present in Mass Spectra of Uric Acid Derivatives

Number	Compound	Transition	Calculated m^*	Found m^*	Fragment Expelled
IX	1,3,7-Trimethyluric acid	210 → 153	111.4	111.5	Methylisocyanate
		153 → 125	102.1	102.5	Carbon monoxide
X	1,3-Dimethyluric acid	196 → 139	98.6	98.5	Methylisocyanate
		139 → 111	88.6	88.8	Carbon monoxide
		111 → 83	62.1	62.5	Carbon monoxide
XI	1,7-Dimethyluric acid	196 → 153	119.4	119.5	Isocyanic acid
		196 → 139	98.6	98.5	Methylisocyanate
		139 → 111	88.6	88.8	Carbon monoxide
XII	3,7-Dimethyluric acid	196 → 153	119.4	119.5	Isocyanic acid
		153 → 125	102.1	102.2	Carbon monoxide
XIII	1-Methyluric acid	182 → 139	106.1	106.2	Isocyanic acid
		182 → 125	85.9	85.7	Methylisocyanate
		125 → 97	75.2	75.4	Carbon monoxide
XIV	3-Methyluric acid	182 → 139	106.1	106.2	Isocyanic acid
		139 → 111	88.6	88.5	Carbon monoxide
XV	7-Methyluric acid	182 → 139	106.1	106.2	Isocyanic acid
		139 → 111	88.6	88.8	Carbon monoxide
XVI	Uric acid	168 → 125	93.0	93.2	Isocyanic acid
		125 → 97	75.2	75.3	Carbon monoxide

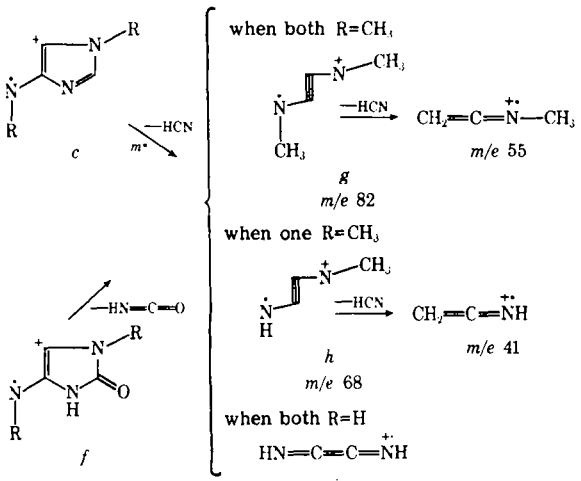


d



e *f*

Scheme II



c *f*

Scheme III

1-*N*-methyl substituent, which is followed by the elimination of a molecule of carbon monoxide. The resulting radical ion *c* in the methylxanthine series then loses a molecule of hydrogen cyanide, while the analogous radical ion *f* from the methyluric acid derivatives expels a molecule of isocyanic acid. The nature of the fragmentation products resulting from the radical ions *c* and *f* is dictated by the number of *N*-methyl substituents present; the species formulated as *g*, *h*, and *i* are formed depending upon whether the fragmenting radical ion is di-*N*-methylated, mono-*N*-methylated, or unsubstituted.

Thus, mass spectrometry is a convenient and rapid method for the identification of caffeine and its possible metabolites formulated as methylated xanthine and uric acid derivatives. Furthermore, microgram quantities of these compounds isolated from biological materials by TLC as single bands (5) could be directly identified by mass spectrometry without derivatization or additional purification.

The major mass spectral fragment peaks and their relative intensities useful in the identification of the 16 compounds studied are given in Tables I and II. The metastable ions in the mass spectra (Tables III and IV) provide additional data in support of the identification made on the basis of fragmentation peaks and molecular ions.

EXPERIMENTAL

All mass spectra were recorded on a GC–mass spectrometer[1] at 70 ev. electron beam voltage. Solid samples were introduced into the ionizing chamber with the aid of a direct insertion probe. The probe was heated to vaporize the solid samples, and mass spectra were recorded at the appropriate temperature[2] (Tables I and II) when the sample ions began to appear as indicated by the total ion-current detector. The source was maintained at 290°.

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[1] LKB-9000.

[2] The temperature recorded was regarded only as an indicator of the probe temperature and was read off the thermocouple temperature indicator.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 19, 1972, from the **Laboratory of Chemistry, National Heart and Lung Institute, National Institutes of Health, Bethesda, MD 20014*, and the *†Department of Environmental and Industrial Health, School of Public Health, University of Michigan, Ann Arbor, MI 48104*

Accepted for publication July 5, 1972.

The authors are grateful to Dr. H. M. Fales for encouragement and many helpful suggestions. Investigations at the University of Michigan were supported in part by PHS Grant GM 15269.

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Chemical Constituents of *Cocculus carolinus* D.C. (Menispermaceae)

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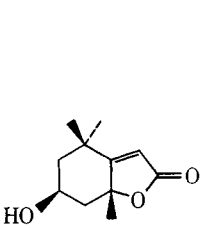
Abstract □ A phytochemical investigation of an ethanolic extract of the stems and leaves of *Cocculus carolinus* D.C. (Menispermaceae) resulted in the isolation and characterization of six compounds: the cyclitols, (+)-quercitol and (−)-viburnitol; the lactone, loliolide; and the alkaloids, sinoacutine, magnoflorine, and palmatine. In each case, the identity of the constituent was confirmed by spectral and mixed melting-point comparison with authentic samples.

Keyphrases □ *Cocculus carolinus* D.C. (Menispermaceae)—isolation and identification of chemical constituents □ (+)-Quercitol—isolated and identified from *Cocculus carolinus* D.C. □ (−)-Viburnitol—isolated and identified from *Cocculus carolinus* D.C. □ Loliolide—isolated and identified from *Cocculus carolinus* D.C. □ Sinoacutine—isolated and identified from *Cocculus carolinus* D.C. □ Magnoflorine—isolated and identified from *Cocculus carolinus* D.C. □ Palmatine—isolated and identified from *Cocculus carolinus* D.C.

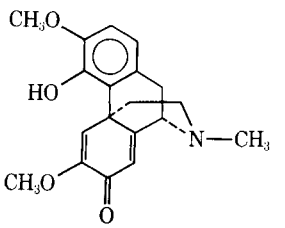
Cocculus carolinus D.C., a species native to the southeastern United States, belongs to a family (Menispermaceae) from which over 99 alkaloid constituents have been isolated (1). In a preliminary phytochemical study, Wall *et al.* (2) indicated the presence of alkaloids in the leaves of this species. Thus, a systematic phytochemical investigation was initiated on this species.

An ethanol extract of the stems and leaves was fractionated into nonquaternary alkaloids (phenolic and nonphenolic), quaternary alkaloids, and acid–neutral fractions. On concentration of the ethanolic extract, a crystalline mixture deposited. Column chromatography of this material on cellulose resulted in the isolation and identification of the cyclitols, (+)-quercitol and (−)-viburnitol. (+)-Quercitol has been previously isolated from several other members of the Menispermaceae: *C. trilobus* D.C. and *C. laurifolius* D.C. (3), *Legnephora moorei* Miers (4), *Cissampelos pareira* L. (5), and *Tiliacora racemosa* Colebr. (6). (−)-Viburnitol has been isolated from several plant families, including two members of the Menispermaceae: *Stephania hernandifolia* Walp. (7) and *Menispermum canadense* L. (8).

Chromatography of the nonquaternary nonphenolic alkaloid fraction resulted in the isolation and characterization of the nonalkaloid, loliolide (I). Loliolide was



I



II

first isolated in 1964 by Hodges and Porte (9) from an ether extract of *Lolium perenne* L. (Graminae). In that same year, Wada and Satoh (10) obtained this same compound from the leaves of *Digitalis purpurea* L. (Scrophulariaceae). In 1969, Pailer and Haschke-Hofmeister (11) isolated loliolide from *Plantago major* L. (Plantaginaceae). This is the first reported occurrence of this compound in the Menispermaceae.

Chromatography of the nonquaternary phenolic alkaloid fraction resulted in the isolation and identification of sinoacutine (II). This alkaloid was first isolated from the Chinese drug "Ching-feng-teng," *Sinomenium acutum* Rehd *et* Wils. (Menispermaceae) (12). Sinoacutine has since been isolated from three other species: *Cassytha pubescens* R.Br. (Lauraceae) (13), *Croton flavens* L. (Euphorbiaceae) (14), and *Corydalis pallida* var. *tenuis* (Fumariaceae) (15). This is the first reported occurrence of this alkaloid in the genus *Cocculus*.

Ion-exchange and adsorption chromatography of the quaternary alkaloid fraction resulted in the isolation and identification of magnoflorine and palmatine.