Experimental Section

The ^1H NMR spectra were recorded on a JEOL C60H spectrometer with 2,2-dimethyl-2-silapentanesulfonic acid, sodium salt as internal reference. Mass spectra were obtained using an LKB-GC/MS Model 9000S (electron impact), a Varian 112S MS (chemical ionization), and a Varian MAT 731MS (field desorption). 1,3-Dimethyl-6-aminouracil was purchased from Het-Chem Co., Harrisonville, Mo.

***N,N'*-Dimethyl-*N*-[3-amino-3-methoxy-2-(3-carbomethoxypropynoyl)acryloyl]urea (5).** To a suspension of 1,3-dimethyl-6-aminouracil (1.55 g, 10 mmol) in $(CH_3)_2SO$ (20 mL) was added dimethyl acetylenedicarboxylate (1.35 mL, 11 mmol). The suspension was stirred at 25 °C for 1 h. Methanol (30 mL) was added. After 8 h at −5 °C, the pale yellow solid was filtered and washed with Et_2O to give 1.68 g (57%) of **5**: MS, *m/e* 265 (EI, CI), 297 (field desorption); ^1H NMR [$(CD_3)_2SO$] δ 9.03 (br d, 1 H, NH), 8.55 (br s, 2 H, NH_2), 3.68 (s, 3 H, OCH_3), 3.57 (s, 3 H, OCH_3), 3.12 (s, 3 H, NCH_3), 2.60 (d, 3 H, NCH_3, J_{H,CH_3} = 4 Hz). The signal appearing at δ ~3.4 arose from H_2O in the solvent.

Anal. Calcd for $C_{12}H_{15}N_3O_6 \cdot 0.5H_2O$: C, 47.05; H, 5.27; N, 13.71. Found: C, 47.35; H, 5.26; N, 13.54.

Acknowledgment. This study was supported by Research Grant 12823 from the National Cancer Institute, NIH. One of us (G.L.A.) wishes to express his appreciation to the American Foundation for Pharmaceutical Education for the Albert H. Diebold Memorial Fellowship, 1974–76. We are grateful to Drs. James A. McCloskey and David Smith for obtaining the mass spectral data reported herein.

Registry No.—**1a**, 32970-29-9; **3**, 6642-31-5; **5**, 63744-45-6; dimethyl acetylenedicarboxylate, 762-42-5.

References and Notes

(1) G. L. Anderson, J. L. Shim, and A. D. Broom, *J. Org. Chem.*, **42**, 993 (1977).
(2) A. D. Broom, J. L. Shim, and G. L. Anderson, *J. Org. Chem.*, **41**, 1095 (1976).
(3) H. Ogura and M. Sakaguchi, *Chem. Pharm. Bull.*, **21**, 2014 (1973).
(4) J. L. Shim, R. Niess, and A. D. Broom, *J. Org. Chem.*, **37**, 578 (1972).
(5) D. V. Santi and A. L. Pogolotti, Jr., *J. Heterocycl. Chem.*, **8**, 265 (1971).
(6) B. A. Otter and J. J. Fox, *J. Am. Chem. Soc.*, **89**, 3663 (1967).
(7) Y. F. Shealy and C. A. O'Dell, *J. Heterocycl. Chem.*, **13**, 1041 (1976).

Identification of Alkaloids in Crude Extracts by Mass-Analyzed Ion Kinetic Energy Spectrometry

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Received May 13, 1977

Complex mixtures can be analyzed by a new method[2] based upon ion kinetic energy measurements. We show here that this procedure, which does not require chromatography and involves minimal sample pretreatment, is applicable to the identification of alkaloids in crude plant extracts.

The procedure involves the following steps. (i) The mixture is ionized by electron impact (EI) or by chemical ionization (CI). (ii) An ion of interest, usually the molecular ion or the protonated alkaloid, is selected by mass analysis. (iii) The mass-analyzed ion is excited by collision which causes it to fragment. (iv) The fragments are identified by kinetic energy analysis. Mass-analyzed ion kinetic energy (MIKE) spectra were obtained in this way for selected ions from crude extracts of the cacti *Dolichothele longimamma* (DC.) Br. and R., *Dolichothele uberiformis* (Zucc.) Br. and R., *Lophophora williamsii* (Lem.) Coult., and *Opuntia spinosior* (Eng.) Toumey. Alkaloid structures were deduced either directly from these spectra in the cases of new alkaloids or by comparison with MIKE spectra of authentic alkaloids.

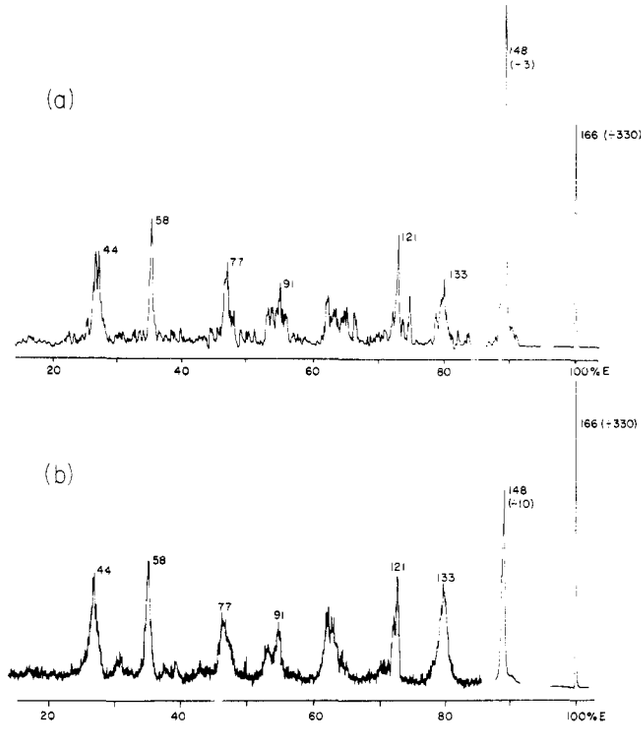


Figure 1. MIKE spectra of *m/e* 166 obtained from (a) a crude *D. longimamma* extract and (b) ubine hydrochloride. The major fragment ions are indicated on the spectra and their origins explained in Scheme I.

Experimental Section

The MIKE spectrometer has been described elsewhere.[3] Samples were introduced from a direct insertion probe at a source temperature (100–200 °C) appropriate for evaporation of the component of interest. Chemical ionization reagent gases were methane or isobutane as indicated. The ion accelerating voltage was 7 kV, the electron emission current was 0.1–0.2 mA (CI and EI), and the indicated pressure of collision gas (always introduced for these studies) was 5 × 10^{-5} Torr.

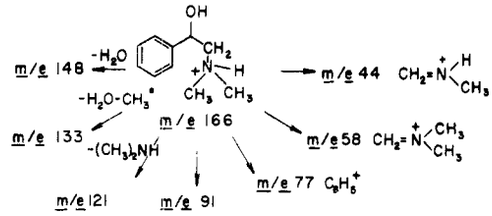
The alkaloid extract used in the EI study was the phenolic fraction obtained from *D. uberiformis* as described elsewhere.[4] The other extracts were obtained from 1 g of freeze-dried cactus from which lipids were removed by overnight Soxhlet extraction with cyclohexane. Extraction with chloroform–methanol–ammonium hydroxide (2:2:1) and evaporation yielded an alkaloid-containing mixture which was analyzed without further work-up. Only a small portion of the extract was used in the analysis.

Results and Discussion

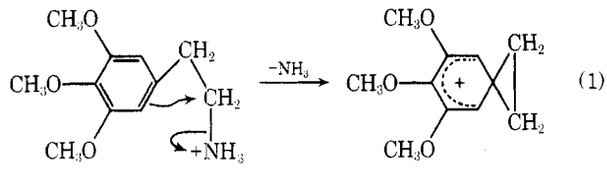
The power of the ion kinetic energy method can be illustrated by the identification of ubine (1) in *D. longimamma*. Studies by traditional chromatographic and spectroscopic methods[5] revealed a number of new as well as previously known alkaloids in this plant not including ubine. The CI (isobutane) mass spectrum of the plant extract shows an ion which corresponds in mass to protonated ubine (*m/e* 166). The MIKE spectrum of this ion (Figure 1a) was interpreted as requiring the ubine structure for the alkaloid. Scheme I summarizes the fragmentation pattern upon which this assignment was based. The MIKE spectrum of authentic protonated ubine (Figure 1b) confirmed the assignment. It is noteworthy that these results were obtained in a few hours using a very crude plant extract. Other constituents of *D. longimamma* studied in this way will be discussed elsewhere.

Our procedure can be used in a survey of plant materials for

Scheme I. Major Collision-Induced Fragmentations of Protonated Ubine



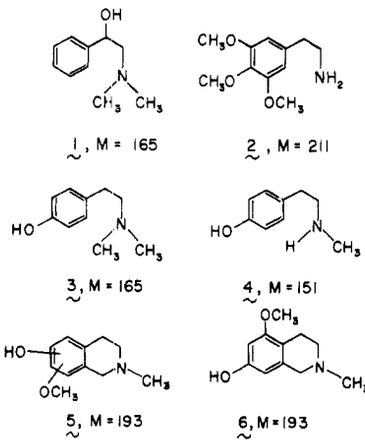
alkaloids of particular interest as well as in the characterization of new alkaloids. The occurrence of mescaline (2) in cactus extracts provides a case in point. The MIKE spectrum of protonated mescaline is characterized by an intense peak due to elimination of a fragment of 17 mass units. It is suggested that reaction 1 occurs as shown. The double analysis inherent



(1)

in the MIKES method, viz. analysis for the mass of the precursor ion as well as that of the fragments, makes the method highly specific. Mescaline could, therefore, be identified in *O. spinosior* simply on the basis of its molecular weight (211) and its fragmentation by loss of NH_3 from the protonated species. The MIKE spectrum of a peyote extract was studied to confirm this assignment. Further confirmation of the presence of mescaline in *O. spinosior* was obtained by comparing the MIKE spectra of the *fragment* ion due to NH_3 loss with that for the same ion in authentic mescaline hydrochloride and in peyote extracts.

The foregoing studies were made by chemical ionization. Electron impact ionization is also useful in this type of study although the resulting mass spectra can show extensive fragmentation which is a disadvantage in recognizing molecular ions. On the other hand, MIKE spectra obtained on molecular ions compare well with electron impact mass spectra of the pure compounds and this facilitates structural assignments. Hordenine (3) and *N*-methyltyramine (4) were identified in the *D. uberiformis* extract on the basis of the MIKE spectra shown in Table I. For comparison the electron impact mass spectra of the pure compounds are also shown.



1, M = 165 2, M = 211

3, M = 165 4, M = 151

5, M = 193 6, M = 193

Electron impact also showed the presence of a new alkaloid, molecular weight 193 in *D. uberiformis*. The MIKE spectrum of this alkaloid showed fragments formed by loss of 1, 15, 17, and 43 mass units. This was interpreted[6] as corresponding to the structure 5 in which the positions of the aryl substituents were not established. In independent work[4] the new alkaloid

Table I. Fragment Ions in Mass and MIKE Spectra

Hordenine (3)		*N*-Methyltyramine (4)	
Mass spectrum	MIKES	Mass spectrum	MIKES
121 (0.05)	(0.05)	149 (0.02)	
120 (0.04)	(0.01)	121 (0.03)	(0.01)
107 (0.09)	(0.05)	120 (0.05)	(0.01)
91 (0.08)	(0.01)	108 (0.06)	(0.13)
77 (0.15)	(0.02)	107 (0.11)	(0.07)
58 (1.00)	(1.00)	91 (0.03)	
		78 (0.02)	(0.01)
		77 (0.08)	(0.02)
		58 (0.42)	(0.01)
		44 (1.00)	(1.00)

uberine (6) was isolated from this plant and its structure was established by conventional methods.

In conclusion, the ion kinetic energy method of mixture analysis has been shown to be applicable to the identification and structural elucidation of alkaloids in crude plant extracts. The method represents an alternative to GC/MS and has comparable sensitivity and specificity. It may be particularly appropriate in studies of alkaloids and other involatile compounds for which gas chromatography is difficult.

Acknowledgment. This work was supported by the National Science Foundation and the National Institute of General Medical Sciences (GM 21,211). T.L.K. acknowledges partial support from the Ball State Special Leave Program.

Registry No.—1, 34469-09-5; 2, 54-04-6; 3, 539-15-1; 4, 370-98-9; 5, 63715-57-1; 6, 63596-58-7.

References and Notes

(1) (a) Department of Chemistry; (b) Department of Medicinal Chemistry and Pharmacognosy.
(2) T. L. Kruger, J. F. Litton, R. W. Kondrat, and R. G. Cooks, *Anal. Chem.*, **48**, 2113 (1976).
(3) J. H. Beynon, R. G. Cooks, J. W. Amy, W. E. Baitinger, and T. Y. Ridley, *Anal. Chem.*, **45**, 1023(A) (1973). See also J. F. Litton, Ph.D. Thesis, Purdue University, 1976.
(4) R. L. Ranieri and J. L. McLaughlin, *Lloydia*, **40**, 173 (1977).
(5) R. L. Ranieri and J. L. McLaughlin, *J. Org. Chem.*, **41**, 319 (1976).
(6) The intense H· loss suggests the tetrahydroisoquinoline structure; loss of 43 (CH_3· + CO) is common to aryl methyl ethers and corresponds here also to retro Diels–Alder fragmentation.

Synthetic Studies on the Side Chains of Cephalotaxus Esters

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Received May 19, 1977

The naturally occurring antileukemic esters of cephalotaxine (I), e.g., isoharringtonine (IIa) and deoxyharringtonine (IIb), have proven to be formidable synthetic objectives due to steric problems in attaching the side chain to cephalotaxine;[1] in fact, the only reported success involved esterification with an α-keto acid (good yield) followed by addition to the keto group (very low yield) to give IIb.[2] It was also possible to esterify cephalotaxine (I) with two other acids with sp^2 hybridized α carbons: *p*-bromobenzoic acid[3] (80% yield) and half-ester IVa[4] (57% yield). We have independently been working on approaches to half-esters IIIa and Va in the hope that they might be sufficiently sterically unhindered to combine with cephalotaxine (I) to form esters which could be further transformed into IIa and IIb and wish to report more efficient routes to compounds in the III, IV, and V series than