The Alkaloids of *Aspidosperma neblinae*. An Application of a Directly Coupled Gas Chromatograph-Mass Spectrometer

The plant species *Aspidosperma neblinae* contains neblinine as its major alkaloidal constituent which can be obtained by crystallization from the crude bases. Its structure (**11**) has been elucidated[1], but the minor alkaloids of this plant had not been investigated. A sample (390 mg) of the extract, from which neblinine had been removed, was at our disposal, but it seemed that little could be done with this small amount of material without the application of special techniques.

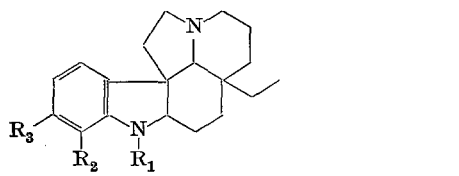
Previous work in this laboratory had indicated the potential usefulness of a gas chromatograph directly coupled to a high resolution mass spectrometer for the analysis of complex alkaloid mixtures[2]. More recently we have developed a combination of a simple commercial gas chromatograph with a medium resolution mass spectrometer[3], and have considerably improved the performance of the system, particularly with respect to the gas chromatographic resolution of complex molecules of relatively low volatility. The investigation of the alkaloid extract of *A. neblinae* is discussed here as an example.

The extract[4] was first chromatographed on alumina (Woelm II) from which benzene/chloroform (9:1) eluted 148 mg of material. Elution with chloroform gave 154 mg of alkaloids which included deacetylpyrifolidine (**2**), 1,2-dehydrodeacetylpyrifolidine (**9**), aspidospermine (**4**), pyrifolidine (**6**), and aspidocarpine (**7**), as indicated by the gas chromatogram of this fraction which is shown in Figure 1. The benzene/chloroform eluate was rechromatographed on alumina from which benzene/petroleum ether (2:1) eluted eburnamonine (**10**) and demethylaspidospermine (**5**). Elution with benzene provided aspidospermidine (**1**), 1,2-dehydroaspidospermidine (**8**), deacetylpyrifolidine (**2**), 1,2-dehydrodeacetylpyrifolidine (**9**), demethoxyaspidospermine (**3**), demethylaspidospermine (**5**), and neblinine (**11**). Elution with benzene/methanol (99:1) gave additional deacetylpyrifolidine and its 1,2-dehydro derivative, and pyrifolidine and aspidocarpine (**7**).

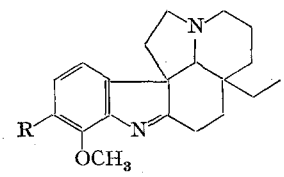
The alumina chromatograms probably were not necessary, considering the excellent resolution of the gas chromatograph, but they had already been done before the GC-MS system was fully developed. The content of each of the alumina fractions was determined by injecting a 10 µg portion of each into the gas chromatograph, and taking rapid oscillograph scans of the mass spectra as each component emerged, as indicated by the flame ionization detector. All compounds obtained, with the exception of neblinine and eburnamonine, belonged to the aspidospermine class of alkaloids, and the identification of each was readily possible by the characteristic aspidospermine fragmentation patterns[5].

The results are summarized in the Table, which indicates the compounds identified and their concentrations in our alkaloid sample.

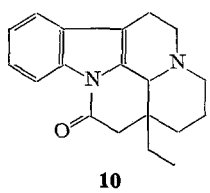
1,2-dehydroaspidospermidine (**8**) and 1,2-dehydrodeacetylpyrifolidine (**9**) were not completely separated



1: $R_1, R_3 = H$; $R_2 = OCH_3$
2: $R_1 = H$; $R_2, R_3 = OCH_3$
3: $R_1 = COCH_3$; $R_2, R_3 = H$
4: $R_1 = COCH_3$; $R_2 = OCH_3$; $R_3 = H$
5: $R_1 = COCH_3$; $R_2 = OH$; $R_3 = H$
6: $R_1 = COCH_3$; $R_2, R_3 = OCH_3$
7: $R_1 = COCH_3$; $R_2 = OH$; $R_3 = OCH_3$



8: R = H
9: $R = OCH_3$



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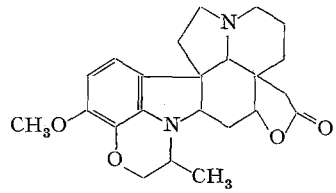

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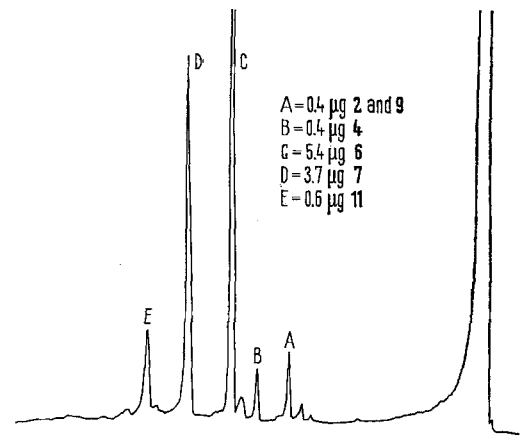

Fig. 1. Gas chromatogram of the chloroform eluate of *A. neblinae*.

[1] K. S. Brown and C. Djerassi, J. Am. chem. Soc. *86*, 2451 (1964).
[2] J. T. Watson and K. Biemann, Analyt. Chem. *37*, 844 (1965).
[3] Gas chromatography was done with a Varian Aerograph HyFl Model 600D gas chromatograph, with a Model 326 linear temperature programmer. Glass columns, 5 ft × 1/8 in. were packed with 1% SE-30 on 100–200 mesh Gas-Chrom Q. Each chromatogram was programmed from 150–250° at 8°/min, and samples of 10 µg in 10 µl of methanol were introduced into a glass-lined injector at 300°. A Hitachi Perkin-Elmer mass spectrometer, Model RMU-6D, was used with 3 sec scans over the range *m/e* 14–500. A pressure reduction system[2], maintained at 210°, was connected to a 'T' at the flame detector base by 8.9 in. of 0.0075 in. I.D. stainless steel tubing at 250°, which gave approximately a 1:1 split of column effluent between the flame detector and pressure reduction unit. We are indebted to Mr. R. Murphy for the construction of this system.
[4] We thank Dr. M. Gorman (Eli Lilly and Co.) for the contribution of this extract.
[5] K. Biemann, M. Spiteller-Friedmann and G. Spiteller, J. Am. chem. Soc. *85*, 631 (1963).

from their corresponding 1, 2-saturated compounds. However, successive mass spectral scans while each compound was emerging from the gas chromatograph showed partial separation (the dehydro compounds have a slightly longer retention time) and their structures were obvious from the peaks at M-29 and M-70[11].

The alkaloids of *A. neblinae*

	% of extract
Aspidospermidine (**1**)[5]	0.1
1,2-Dehydroaspidospermidine (**8**)[5]	
Deacetylpyrifolidine (**2**)[6]	3.3
1,2-Dehydrodeacetylpyrifolidine (**9**)	
Demethoxyaspidospermine (**3**)[7]	0.9
Aspidospermine (**4**)[8]	1.3
Demethylaspidospermine (**5**)[7]	4.7
Pyrifolidine (**6**)[6]	26.3
Aspidocarpine (**7**)[9]	18.7
Eburnamonine (**10**)[10]	0.05
Neblinine (**11**)[1]	0.8

A typical example is shown in Figure 1, which represents the gas chromatogram of the chloroform eluate of the alumina column. The weights indicated in this figure are the quantity of each component in one 10 μg injection. The mass spectra of 2 of these components, aspidospermine (**4**) and pyrifolidine (**6**), are reproduced in the Figures 2 and 3, respectively. Even 0.4 μg of aspidospermine present in this mixture was sufficient to give an excellent mass spectrum. The peaks at *m/e* 147, 207, and 281 in the spectra of these figures are due to products from the liquid phase of the gas chromatograph column. These peaks were identified as such by the recording of a background spectrum between chromatographic peaks; they do not hinder the interpretation of the mass spectra of the alkaloids, but in fact are found useful as mass markers in the case of a very weak, otherwise uncountable spectrum.

Such a system enables one to speedily separate crude alkaloid mixtures and simultaneously obtain the mass spectra of the components and thus lends itself well for detailed screening of such extracts and the investigation of the above mentioned sample shall serve as an example[12].

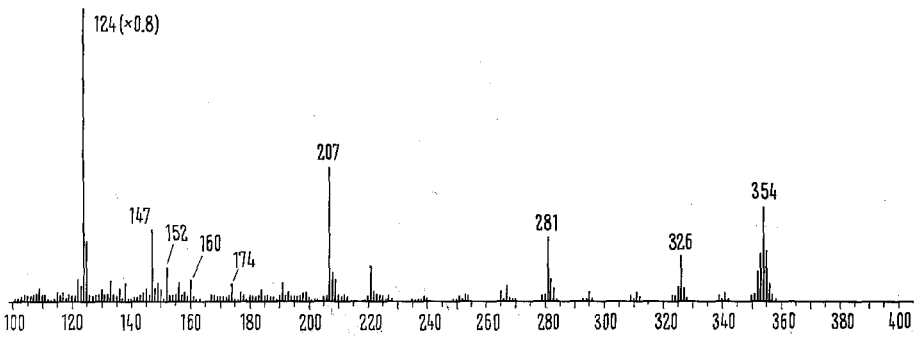

Fig. 2. Mass spectrum of component B (aspidospermine) of Figure 1.

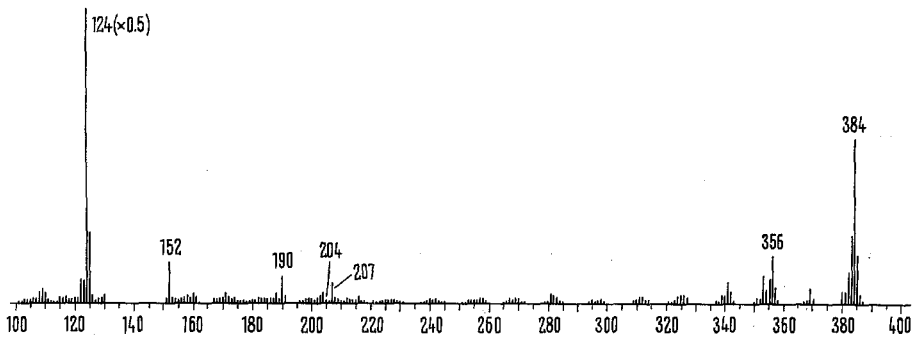

Fig. 3. Mass spectrum of component C (pyrifolidine) of Figure 1.

[6] C. DJERASSI, B. GILBERT, J. N. SHOOLERY, L. F. JOHNSON and K. BIEMANN, Experientia *17*, 162 (1961).
[7] J. M. FERREIRA, B. GILBERT, R. J. OWELLEN and C. DJERASSI, Experientia *19*, 585 (1963).
[8] J. F. D. MILLS and S. C. NYBURG, J. chem. Soc. *1458* (1960).
[9] S. MCLEAN, K. PALMER and L. MARION, Can. J. Chem. *38*, 1547 (1960).
[10] M. F. BARTLETT and W. I. TAYLOR, J. Am. chem. Soc. *82*, 5941 (1960).
[11] K. BIEMANN and G. SPITELLER, J. Am. chem. Soc. *84*, 4578 (1962).
[12] This work was supported by a grant from the National Science Foundation No. GP 7166.
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Zusammenfassung. Die Analyse der Basenfraktion von *Aspidosperma neblinae* mit Hilfe eines mit einem Massenspektrometer gekuppelten Gas-Chromatographen führte zur Identifizierung der folgenden Alkaloide: Aspidospermidin, 1, 2-Dehydroaspidospermidin, Deacetylpyrifolidin, 1, 2-Dehydrodeacetylpyrifolidin, Demethoxyaspidospermin, Aspidospermin, Demethylaspidospermin, Pyrifolidin, Aspidocarpin, Eburnamonin, und Neblinin.

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