MASS SPECTRA OF LYCOCTONINE-TYPE DITERPENOID ALKALOIDS FROM *DELPHINIUM AJACIS* AND THEIR DERIVATIVES[1]

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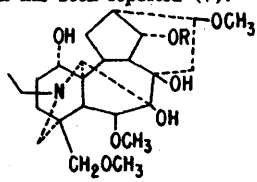
Three lycoctonine-type diterpenoid alkaloids (delcosine, acetyldelcosine and delsoline) isolated from *Delphinium ajacis* seeds were analyzed by low and high resolution mass spectrometry. The common mode of fragmentation was the formation of the M − 189 fragment ion and the loss of peripheral functional groups. The trimethylsilyl and acetyl derivatives mainly exhibited the loss of peripheral functional groups and facilitated analysis by gas-liquid chromatography.

Diterpenoid alkaloids isolated from the common larkspur, *Delphinium ajacis*, can be classified into two distinct structural types of compounds, the lycoctonine type, represented by delcosine, acetyldelcosine and delsoline, and the atisine type, represented by ajaconine. In an earlier communication (1) the biosynthesis of these alkaloids from mevalonate-2-^{14}C and glycine-2-^{14}C was reported. At that time only delcosine was conclusively identified from the complex mixture of alkaloids obtained from the mature plants, whereas the seed yielded ajaconine (2), delcosine (3, 4), acetyldelcosine (3), and several alkaloids of unknown structure. The compound designated earlier as LBA-III (4, 5) has now been conclusively identified as delsoline (6) by infrared, NMR, and mass spectral comparison with an authentic sample. This paper describes the decomposition exhibited upon electron impact of three lycoctonine-type alkaloids and their trimethylsilyl and acetyl derivatives. A preliminary communication has been reported (7).



R = H; DELCOSINE

R = $COCH_3$ ACETYLDELCOSINE

R = CH_3, DELSOLINE

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METHODS

Reagents. Reagent grade *N,O*-bis(trimethylsilyl)acetamide-d_{18} (BSA-d_{18}) (99 atom %) and trimethylchlorosilane (TMCS) (99 atom %) were purchased from Merck, Sharp and Dohme of Canada, Ltd., Montreal, Canada, and the protium form of the reagents from Analabs, Inc., Hamden, Connecticut.

Isolation of alkaloids. The alkaloids were isolated from seeds of *Delphinium ajacis* by extraction and column chromatography on neutral alumina. Final purification was done by TLC (benzene:ethyl acetate:diethylamine, 7:2:1) and crystallization according to the published procedures (1).

Silylation procedure (8). BSA (0.1 ml) and a drop of TMCS were added step-wise to the alkaloid (0.01 g) in a vial. The sealed reaction mixture was kept at 55 C for 3 hr and then the excess reagent was removed by passing a stream of nitrogen over it. The residue was dissolved in acetone and analyzed by GC-MS using a solid injection system (9).

Acetylation procedure. Acetic anhydride (Reagent Grade) (0.5 ml) and a drop of pyridine were added stepwise to the alkaloid (0.01 g) in a vial. The sealed reaction mixture was kept overnight at 25 C and a drop of water was added to the mixture. The mixture was analyzed by GC-MS using a solid injection system (9).

Mass spectra. A prototype (10, 11) LKB-9000 gas chromatograph-mass spectrometer (LKB Instruments, Inc., Rockville, Maryland) and a CEC-110B mass spectrometer (du Pont Instruments, CEC Analytical

Division, Monrovia, California) were used. The total ion current record served as the GLC tracing on the LKB-9000. A 10-ft x ¼-inch glass column packed with 1% OV-1 on Gas Chrom Q (100-110 mesh) (Applied Science Laboratories, State College, Penn.) and equipped with a solid injection system (9) was used on the LKB-9000.

The LKB mass spectrometer was operated at an electron energy of 70 eV, an accelerator voltage of 3.5 kV, a trap current of 6 μ A, an ion source temperature of 290 C, and a direct probe temperature of 30-55 C. The CEC mass spectrometer was operated at an electron energy of 70 eV, an accelerating voltage of 8 kV, and a direct probe temperature of 160-170 C with an indicated source pressure of 2×10^{-6} mm Hg.

RESULTS AND DISCUSSION

Mass spectral analysis of diterpenoid alkaloids

When crystallized delsoline, delcosine, and acetyldelcosine from *D. ajacis* seeds were analyzed by gas-liquid chromatography-mass spectrometry (GC-MS), the compounds partially underwent dehydration on the GLC column as demonstrated by the emergence from the column of compounds that possessed molecular weights of 18 amu less than the injected alkaloids.

Delcosine, acetyldelcosine, and delsoline were subjected to high resolution mass spectrometic analysis and exhibited similar fragmentation patterns, as shown in Figures 1A, 1B, and 1C. These lycoctonine-type alkaloids did not show many fragment ions in the lower mass range. They characteristically lost the peripheral functional groups. Some of the common fragment ions formed were due to the loss of CH_3, OH, H_2O, OCH_3, CH_3OH, and (H_2O + CH_3). Mass spectral studies of heteratisine (12) and other lycoctonine-type (13) alkaloids also showed the loss of similar peripheral functional groups.

The common mode of fragmentation in the lower mass range among delcosine and its derivatives was the formation of the M^+-189 ion. A mechanism for the formation of the M^+-189 fragment ion for delcosine is proposed in Scheme I. Initial cleavage of the C-1 — C-11 bond alpha to the piperidine ring of delcosine gives rearranged ion *a*. Ion *a* forms ion *b* (*m/e* 438) by a 1, 2 shift of a hydrogen atom to an oxygen accompanied by the loss of a methyl group. This transition was confirmed by a metastable ion at *m/e* 423.4. Other ions of *m/e* 420 might also be formed by the loss of one of the other methyl groups in the molecule. The fission of the C-4 — C-18 bond in ion *b*, along with the loss of the C-7 hydroxyl group as water, gives ion *c* (*m/e* 420). Other structures for fragment ions at *m/e* 420 can be visualized as resulting from the loss of any of the other hydroxyl groups. A metastable ion at *m/e* 402.8 confirms this fragmentation process. Ion *c* loses a fragment of *m/e* 156 to form one of *m/e* 264, which is due to the cleavage of C-5 — C-11 and C-6 — C-7 bonds. In ion *d* there is a double bond at C-10 — C-11 as well as C-17 — C-7. This means that migration of the hydrogen atoms at C-17 and C-10 to C-7 and C-11 might occur. The gain of a hydrogen atom at C-7 is difficult to explain since this position is very far from any carbon bearing a free hydrogen atom. It is not apparent how these rearrangements occur; hence, structure *d* is speculative. No metastable ion was observed for the $c \rightarrow d$ transition, a fact suggesting that it occurred in several steps. Similar types of fragmentation pathways, with the corresponding metastable ions, as shown in Scheme I were also observed in delsoline and acetyldelcoline. The elemental composition of the M^+-189 ion *d*, which is common to all three alkaloids, agrees with the proposed structure.

Mass spectra of trimethylsilyl (TMS) derivatives of diterpenoid alkaloids

The separation of these diterpenoid alkaloids by GLC without dehydration was made possible by preparing their trimethylsilyl (TMS) derivatives. These (TMS) derivatives of diterpenoid alkaloids, prepared by using *N,O*-bis(trimethylsilyl)-acetamide (BSA), trimethylchlorosilane (TMCS), and pyridine, on GLC analysis showed a large number of peaks. *N,O*-bis (trimethylsilyl)trifluoroacetamide (BSFA) derivatives were also prepared (using BSFA in pyridine). Both methods failed to effect complete derivatization of all hydroxyl groups. After using BSA and TMCS in the absence of pyridine, a minimum number of peaks for each alkaloid was observed upon GLC analysis. TMS-d_9 derivatives of the alkaloids were also prepared using BSA-d_{18} (> 99 atom %) and TMCS-d_9 (> 99 atom

%) without pyridine. GC-MS analysis of protium and deuterium TMS derivatives showed peaks corresponding to di- and tri-TMS derivatives for delcosine, indicating that the C-1 OH, C-14 OH and either C-8 OH or C-7 OH groups were derivatized. Acetyldelcosine-TMS yielded two peaks corresponding to the mono- and di-TMS derivatives. Hence, when the C-14 OH is blocked by the acetyl group, only C-1 OH and either C-8 OH or C-7 OH remain possibilities for derivatization. Delsoline modified with TMS gave, mainly, a single peak which corresponded to the mono-TMS derivative and a di-TMS derivative in the deuterated sample, an unexpected observation in view of the nearly equal intensities of peaks observed with the acetyldelcosine derivatives. Their masses and corresponding mass shifts found in the deuterated derivatives are shown in Table 1. The change from acetyl to methyl cannot change the reactivity of the C-7 or C-8 hydroxyl groups. The higher amount of the di-TMS derivative of acetyldelcosine is probably formed as a result of hydrolytic removal of some of the acetyl group and subsequent silylation at the C-14 hydroxyl group. The formation of the mono-TMS derivative of delsoline becomes clear since hydrolysis of an ether would not occur as easily as that of an acetate; however, no explanation for the large amount of the di-TMS-d_9 derivative can now be made. This leaves the formation of significant amounts of tri-TMS delcosine also difficult to explain.

TABLE 1. *Molecular weight of TMS and TMS-d, derivatives of lycoctonine-type diterpenoid alkaloids.*

Compound	No. of GLC peaks	TMS Peak 1	TMS-d_9 Peak 1	TMS Peak 2	TMS-d_9 Peak 2
Delcosine (M+ 453)	2	597	615	669	696
Acetyldelcosine (M+ 495)	2	567	576	639	657
Delsoline (M+ 467)	1	539	548[a]	611[a]	629

[a] Not observed in significant amounts

Mass spectra of the protium TMS derivatives

TABLE 2. *Mass spectral relative intensities of protium trimethylsilyl derivatives of three lycoctonine-type diterpenoid alkaloids.*

	Delcosine di TMS M+ 597	Delcosine tri TMS M+ 669	Acetyldelcosine mono TMS M+ 567	Acetyldelcosine di TMS M+ 639	Delsoline mono TMS M+ 539
M+	trace	trace	5	3	trace
M+−15	20	5	14	5	8
M+−17	100	25	100	30	85
M+−18	8	44	5	100	28
M+−31	—	—	—	10	10
M+−33	10	18	18	—	100
M+−35	40	—	30	—	85
M+−90	10	3	8	—	8
M+−107	5	30	4	72	50
M+−108	18	3	14	—	5

TABLE 3. *Mass spectral relative intensities of trithylsilyl (d_9) derivatives of three lycoctonine-type diterpenoid alkaloids*

	Delcosine di TMS (d_{18}) M+ 615	Delcosine tri TMS (d_{27}) M+ 696	Acetyldelcosine mono TMS (d_9) M+ 576	Acetyldelcosine di TMS (d_{18}) M+ 657	Delsoline di TMS (d_9) M+ 629
M+	trace	trace	trace	trace	trace
M+−17	50	10	100	50	75
M+−18	100	35	10	10	18
M+−19	25	10	5	8	10
M+−20	12	6	8	12	15
M+−31	4	4	—	20	4
M+−33	12	20	10	4	10
M+−35	31	—	25	—	—
M+−115	6	15	—	12	30
M+−116	18	5	12	40	75
M+−117	—	3	—	—	4

tives of alkaloids (Table 2) and of the deuterated TMS derivatives (Table 3) exhibited patterns similar to those of the parent alkaloids. The common fragment ions were due to loss of the following groups: CH_3, OH, H_2O, OCH_3, (CH_3 + OH), TMSOH, and (OH + OTMS). The fragment ions M^+-18, M^+-19, and M^+-20 in deuterated TMS derivatives were formed by the loss of DO, DHO and D_2O respectively. That at M^+-17 was due to loss of OH from either the C-8 and/or C-7 tertiary carbon atoms. Preparation of TMS derivatives was mainly useful as a means to analyze the alkaloids by gas chromatography. This technique may prove valuable in the analysis of crude mixtures of alkaloids isolated from various *Delphinium* species and of diterpenoid alkaloids produced by *Aconitum* and *Garrya* species.

Mass spectra of acetyl derivatives of diterpenoid alkaloids

Delcosine, acetyldelcosine, and delsoline were also converted into the corresponding acetates. On GC-MS analysis acetylated delcosine showed three diacetate GLC peaks, and acetyldelcosine and delsoline two monoacetate GLC peaks each. Since more than one peak for each type of derivative was observed, it was concluded that different hydroxyl groups were esterified; however, it was not possible to determine which hydroxyl groups were acetylated in the individual derivatives. The different locations of esterification and the stereochemistry of different hydroxyl groups explain the different retention times observed. The mass spectra (Table 4) of the three delcosine diacetates and the two acetyldelcosine monoacetates showed similar fragmentation patterns with differences in relative intensities. The common modes of fragmentation of the acetates were due to the loss of CH_3, H_2O, OCH_3, (CH_3 + H_2O), $OCOCH_3$, $HOCOCH_3$, (H_2O + $COCH_3$), (CH_3 + $HOCOCH_3$), (H_2O +

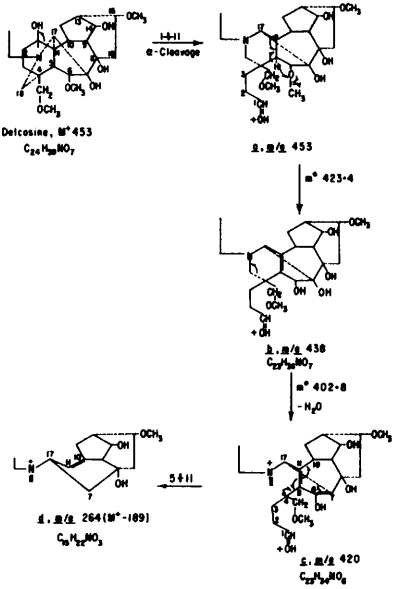

FIGURE 1. Proposed route of formation of the M^+-189 ion.

TABLE 4. *Principal fragment ions and their relative intensities of three* Delphinum *diterpenoid alkaloid acetates.*

	Delcosine diacetate-1 M+ 537	Delcosine diacetate-2 M+ 537	Delcosine diacetate-3 M+ 537	Acetyl delcosine mono-acetate-1 M+ 537	Acetyl delcosine mono-acetate-2 M+ 537	Delsoline mono-acetate-1 M+ 509	Delsoline mono-acetate-2 M+ 509
M+	3	3	1	trace	10	22	1
M+−2	—	50	—	—	8	64	—
M+−15	10	3	1	1	38	2	—
M+−18	5	18	6	100	30	35	12
M+−31	7	8	—	25	36	60	10
M+−33	12	10	—	12	50	26	8
M+−45	—	32	15	—	6	—	20
M+−59	100	40	20	—	40	20	8
M+−60	10	10	50	20	30	20	30
M+−61	5	36	8	20	28	50	100
M+−75	8	10	4	15	25	24	6
M+−77	10	20	20	80	30	20	25
M+−78	4	8	25	30	8	—	35
M+−89	4	50	15	2	6	44	12

$HOCOCH_3$), and (CH_3 + CH_3 + $COCH_3$).

For rapid GLC analysis of lycoctonine alkaloids the TMS derivatives were more helpful than the acetates. A column temperature of 290 C was required for the analysis of the acetates compared to 220 C for TMS derivatives.

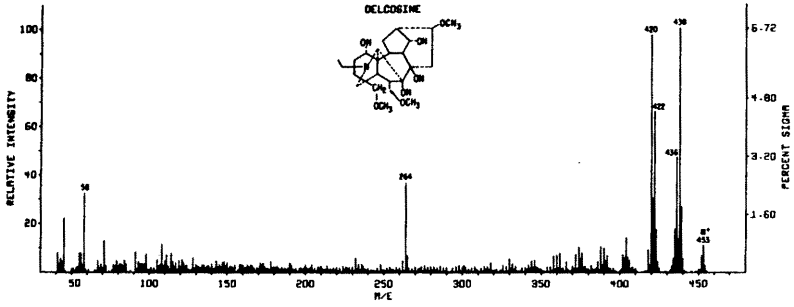

FIGURE 2. Mass spectrum of delcosine.

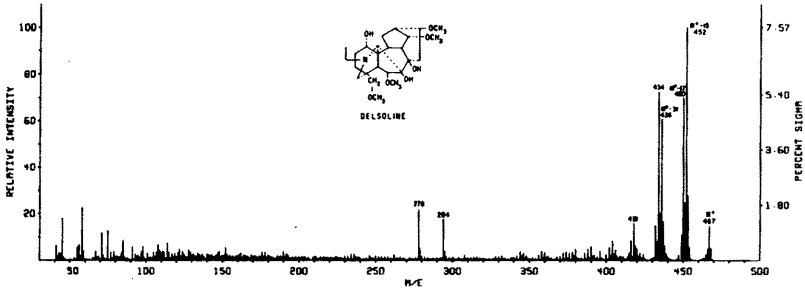

FIGURE 3. Mass spectrum of delsoline.

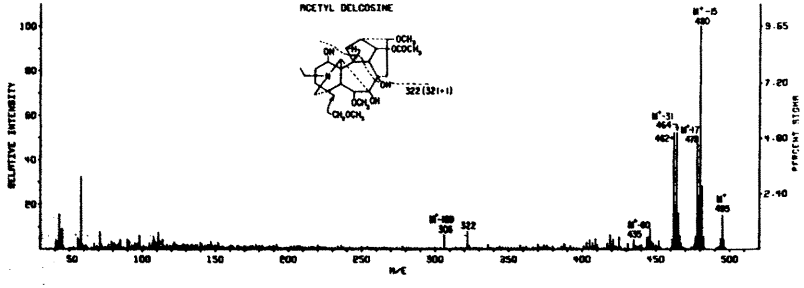

FIGURE 4. Mass spectrum of acetyldelcosine.

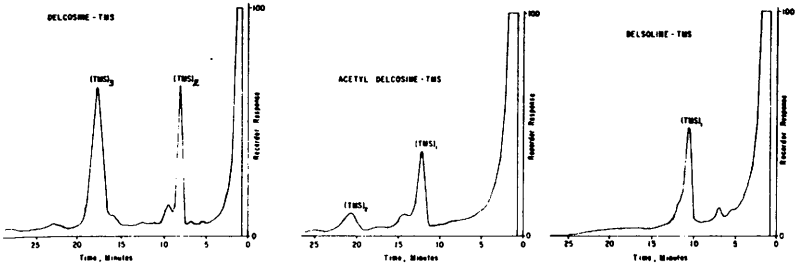

FIGURE 5. MS-GC analysis of *D. ajacis* alkaloids using the solid injection technique (9) (10-ft x ¼-in glass column; 1% OV-1 on 100/110 mesh Gas Chrom Q, flash heater 312 C, molecular separator 280 C, He flow 21 ml/min, spectra taken at 70 eV).

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