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CHEMICAL-IONIZATION MASS SPECTRA OF DITERPENE ALKALOIDS

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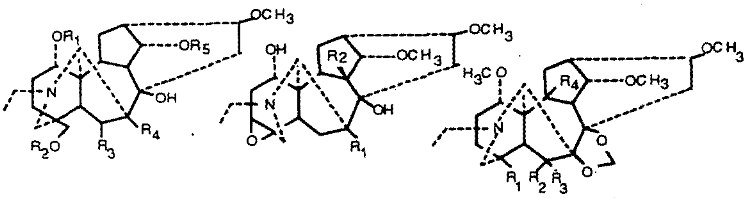
The chemical-ionization spectra of diterpene alkaloids have been investigated for the first time with the recording of positive and negative ions [CI(+) and (−)]. A comparison has been made of the CI(+) spectra with the EI and SIMS spectra. It has been shown that the nature of the CI(+) and (−) spectra depends greatly on the type and position of substituents in the lycoctonine skeleton.

With the aim of a more complete determination of the mass-spectral properties of diterpene alkaloids [1-3], we have studied the chemical-ionization (CI) mass spectra of this series of compounds with the recording of both positive and negative ions. There is no information in the literature on the CI of diterpene alkaloids. We made use of a collection of lycoctonine bases, which were divided into four groups: 1) 8-monools; 2) 6-OMe-7,8-diols; 3) C_{18}-alkaloids with a 3,4-epoxy group; and 4) bases with a 7,8-methylenedioxy group (MDOG).

The spectra of 19 alkaloids were recorded in the CI(+) regime. The reagent gas used was methane. Table 1 gives the relative intensities of the key ions of the CI(+) spectra. Common for all the spectra is the presence of a triplet of ions in the molecular region: $(M + H)^+$, M^+, $(M - H)^+$ but the intensities of the components varied greatly not only on passing from group to group but also within subgroups. The peaks of characteristic ions in the EI [2-4] and SIMS [5] spectra were also present in the CI(+) spectra. However, the relative intensities of the key fragments were different.



1-8,20 9, 10, 21 11-19

1. $R_1=R_3=R_4=R_5=H$. $R_2=CH_3$
2. $R_1=R_2=CH_3$, $R_3=R_4=R_5=H$
3. $R_1=R_5=H$. $R_2=CH_3$, $R_3=OCH_3$, $R_4=OH$
4. $R_1=H$, $R_2=R_5=CH_3$, $R_3=OCH_3$, $R_4=OH$
5. $R_1=R_2=CH_3$, $R_3=OCH_3$, $R_4=OH$, $R_5=H$
6. $R_1=R_5=CH_3$, $R_2=H$, $R_3=OCH_3$, $R_4=OH$
7. $R_1=R_2=R_5=CH_3$, $R_3=OCH_3$, $R_4=OH$
8. $R_1=Ac$, $R_2=R_5=CH_3$, $R_3=OCH_3$, $R_4=OH$
9. $R_1=R_2=H$
10. $R_1=OH$, $R_2=H$
11. $R_1=CH_2OCH_3$, $R_2=R_3=R_4=H$
12. $R_1=CH_2OCH_3$, $R_2+R_3=O$, $R_4=H$
13. $R_1=CH_3$. $R_2+R_3=O$, $R_4=OH$
14. $R_1=CH_2OCH_3$, $R_2=OH$, $R_3=R_4=H$
15. $R_1=CH_3$, $R_2=R_4=OH$, $R_3=H$
16. $R_1=CH_2OCH_3$, $R_2=OCH_3$, $R_3=R_4=H$
17. $R_1=CH_3$, $R_2=OCH_3$, $R_3=H$, $R_4=OH$
18. $R_1=CH_2OCH_3$, $R_2=OAc$, $R_3=R_4=H$
19. $R_1=CH_3$, $R_2=R_4=OAc$, $R_3=H$
20. $R_1=R_5=Ac$, $R_2=CH_3$, $R_3=OCH_3$, $R_4=OH$
21. $R_1=H$, $R_2=OH$

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*Deceased.

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TABLE 1. Relative Intensities (%) of the Key Ions and Stabilities, *W* (%), of the $(M + H)^+$ Ions in the CI (+) Spectra of Compounds (**1**)-(**19**)

Compound	$W_{(M+H)^+}$	$(M+H)^+$	M^+	$(M-H)^+$	$(M-15)^+$	$(M-17)^+$	$(M-31)^+$	$(M-33)^+$	$(M-45)^+$	$(M-47)^+$	$(M-49)^+$	$(M-61)^+$	Other ions
1	18	50	28	22	6	100	17	10	3	5	12	–	436[$(M-29)^+$,5], 418(7)
2	8	26	25	23	9	83	100	7	5	20	10	11	432[$(M-17)+28]^+$,6
3	10	28	29	15	55	100	15	20	3	7	14	3	464[$(M-17)+28]^+$,8
4	20	74	37	23	52	100	26	23	2	4	22	–	
5	7	19	20	19	24	100	39	12	3	6	14	3	478[$(M-17)+28]^+$,8
6	7	23	30	27	27	100	47	13	5	20	27	7	478[$(M-17)+28]^+$,7
7	7	28	43	35	35	100	75	15	3	12	23	8	418(6)
8	4	10	13	12	12	28	12	10	9	10	10	10	450[$(M-59)^+$,100], 434(19)
9	34	100	40	20	15	65	15	4	5	7	10	6	420[$(M+29)^+$,15]
10	38	100	43	19	20	45	4	4	2	2	5	–	436[$(M+29)^+$,15]
11	11	24	18	20	13	9	100	4	12	5	–	9	478[$(M+15)^+$,7]
12	18	35	18	16	3	–	100	–	3	–	–	9	
13	17	38	16	15	8	7	100	–	5	3	1	5	492[$(M+29)^+$,4], 478(2), 434(15)
14	18	60	28	28	7	4	100	11	11	7	–	16	508[$(M+29)^+$,3], 450(17)
15	14	38	21	21	5	16	100	9	3	6	3	5	436(12)
16	14	47	22	30	25	8	100	7	25	12	2	16	508[$(M+15)^+$,8], 464(32)
17	14	40	22	21	11	12	100	3	8	5	–	9	450[$(M-29)^+$,15]
18	14	36	22	21	4	–	100	–	6	–	–	5	492[$(M-29)^+$,26]
19	6	11	8	11	2	–	100	–	–	–	–	8	490[$(M-59)^+$,31], 460(21)

TABLE 2. Relative Intensities (%) of the Key Ions in the CI(−) Spectra of Compounds (**1-15**, **17**, **20**, and **21**)

Compound	$(M-H)^-$	$(M-H-H_2)$	$(M-15)^-$	$(M-17)$	$(M-18)$	$(M-19)^-$	$(M-30)^-$	$(M-31)^-$	$(M-32)^-$	$(M-33)^-$	$(M-34)^-$	$(M-36)^-$	Other ions
1	100	13	–	12	–	12	–	–	8	30	16	–	439[$(M+32)^-$,14], 372(17), 358(9)
2	100	6	–	17	–	8	–	–	28	61	39	–	453[$(M+32)^-$, 3], 372(24), 357(20), 356(15)
3	100	30	3	14	–	11	–	–	–	–	32	24	490[$(M+37)^-$, 7], 488(21), 485(8), 403(13), 401(20), 387(15), 385(12)
4	100	17	3	18	3	9	–	–	–	–	39	26	499[$(M+32)^-$,3], 419(9), 417(13), 415(12), 403(9), 401(11)
5	67	7	-	17	11	8	–	–	23	–	100	29	419(21), 417(37), 415(25), 403(29), 401(32), 387(29), 385(22)
6	44	7	3	9	8	8	–	–	20	–	100	16	419(14), 417(20), 415(12), 403(28), 401(19), 387(20)
7	84	5	5	26	12	9	–	–	24	6	100	14	433(18), 431(30), 417(24), 415(15)
8	100	7	3	13	6	7	–	–	9	–	59	5	568[$(M+59)^-$,7], 459(11), 450(5), 448(9), 447(8)
9	100	7	5	26	30	10	12	9	21	4	20	11	421[$(M+30)^-$,20], 406(20), 403(18), 343(18), 341(15), 317(37)

TABLE 2 (continued)

Compound	$W_{(M+H)}^{+}$	$(M+H)^+$	M^+	$(M-H)^-$	$(M-15)^-$	$(M-17)^-$	$(M-31)^+$	$(M-33)^+$	$(M-45)^-$	$(M-47)^-$	$(M-49)^-$	$(M-61)^-$	Other ions
10	100	9	3	13	14	7	9	3	12	4	18	11	361(15), 359(16), 357(15), 345(9)
11	6	2	5	6	3	–	–	100	23	10	–	–	495[$(M-32)^-$,8], 493(6), 418(4)
12	24	–	5	14	2	–	100	–	47	11	4	–	431(35), 429(8), 417(18), 415(17)
13	32	14	7	13	5	9	100	–	25	5	8	–	476(15), 419(15), 417(35), 415(14), 403(18), 401(22)
14	21	3	–	8	9	–	15	–	100	4	13	3	509[$(M-30)^-$,3], 431(24), 417(25)
15	10	5	3	11	11	20	32	–	100	8	25	8	509[$(M-45)^-$,13], 495(8), 463(72), 461(22), 417(32), 403(48), 401(31)
17	36	13	3	10	13	–	–	100	34	6	17	–	511[$(M-32)^-$,13], 509(20), 433(76), 431(39), 417(82), 415(43), 401(65)
20	100	11	9	37	16	13	–	12	17	7	64	13	489(20), 487(21), 478[$(M-59)^-$, 13], 476(21), 475(21), 459(25), 452(41)
21	100	7	5	16	21	6	11	–	36	–	29	12	419(3), 359(38), 347(18), 345(17)

In the CI(+) of the 8-monools (**1**, **2**), as also in the EI spectra, the maximum peak was that of the $(M - OR_1)^+$ ion, and the next strongest peak was given by the elimination of the OH group at C-8. Thus, in the spectrum of talatisamine (**2**), $(M - 17)^+$ had an intensity of 83% (Table 1).

The spectra of the 7,8-diols (**3-8**) were characterized by a slight fall in the stability of the protonated molecular ion in the series C_1-OH, C_1-OMe, C_1-OAc, which agrees with Hammond's postulate [6]. As compared with the EI spectra, the CI(+) spectra of the alkaloids of this group were less selective (Table 1). For the 1-hydroxy-, (**3**) and (**4**), and 1-methoxy- bases (**5-7**) the maximum peak in the CI(+) spectrum was $(M-17)^+$, the main source of its formation being the diol chain at C_7-C_8. The splitting out of C_1-OR in the series under consideration became predominating for the C_1-OAc base (**8**). In the spectra of the alkaloids of the second group, the peaks of the $(M-15)^+$ were strongest for the 1-OH- and decreased for the 1-OMe- and 1-OAc-bases (Table 1). An analogous tendency has been observed in the EI spectra [1].

In the CI(+) regime, the C_{18}-3,4-epoxy alkaloids (**9**, **10**) gave a fragmentation pattern practically identical with that obtained by the SIMS method [5]: the most stable ion was $(M + H)^+$ and the second strongest peak was that of the $(M - OH)^+$ ion, followed by the peaks of ions corresponding to the splitting out of $C^{\cdot}H_3$ or $O^{\cdot}CH_3$.

As compared with the third group, the behavior of C_{19}-diterpene alkaloids with a 7,8-MDOG (**11-19**) on chemical ionization with methane had a greater analogy with EI [3]: the maximum peak was that of the $(M - OCH_3)^+$ ion, but the contributions of the molecular and fragmentary ions were 5-7 times greater than in EI. Characteristic for the spectra for this group of bases was the presence of the peak of the even-electron ion $(M - 29)^+$, formed from the MH^+ ion on the elimination of a CH_2O molecule at the expense of the MDOG.

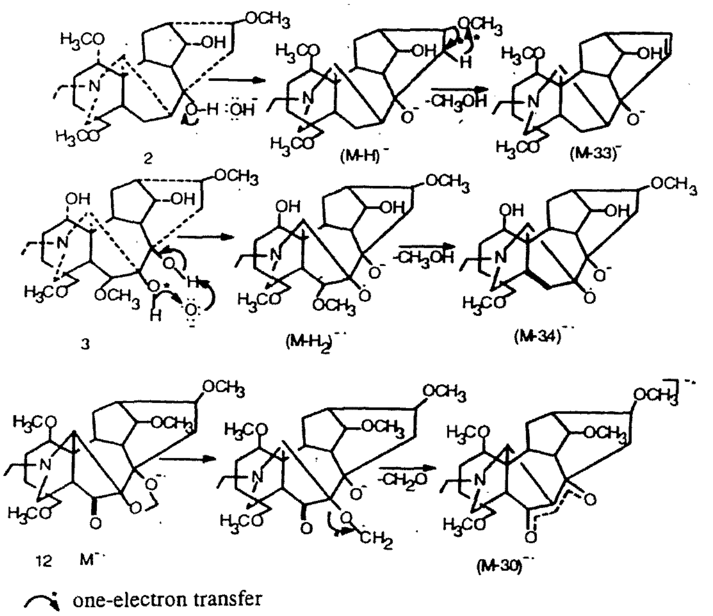
In more than half the CI(+) spectra of the compounds under consideration there were peaks of cluster ions. These were medium-intensity peaks of $(M + 29)^+$ $[MC_2H_5]^+$ ions or, in the spectrum of (**16**) the peak of the $MCH_3{}^+$ ion (Table 1). The spectra of some alkaloids contained the peaks of ions with masses 11 a.m.u. greater than the molecular mass (**1-3**, **5**, **6**, Table 1). The nature of these ions can be represented in the following way: $MC_2H_5{}^+ - H_2O \rightarrow (MC_2H_5 - H_2O)^+$. Also in favor of this is the presence of peaks in the spectra of the bases with the $(M - 17)^+$ ion having an intensity of 100% or close to it.

Thus, the spectra obtained in the CI regime with the recording of positive ions possess a number of characteristic ions: MH^+, M^+, $(M - H)^+$, $(M - 15)^+$, $(M - 17)^+$, and $(M - OCH_3)^+$ and represent an average pattern of the EI and SIMS spectra, but for some subgroups they are closer to EI and for others to SIMS.

In the investigation of the behavior of the diterpene alkaloids in the CI regime with the recording of negative ions, as the reagent gas we used a mixture of methane and nitrous oxide in a ratio of 1:1. When the reagent gas mixture is bombarded with electrons, as a result of the reaction

$$N_2O + e^- \rightarrow N_2 + O^{-\cdot}$$
$$O^{-\cdot} + CH_4 \rightarrow OH^- + \dot{C}H_3$$

the anions $O^{-\cdot}$ and OH^- possessing high proton affinities are formed [7, 8].



A common feature of the CI(−) spectra of the compounds of the first three groups of alkaloids is the presence of the peak of the deprotonated molecular ion as the 100% peak or the second-strongest peak (Table 2). Its formation probably takes place through an ion-molecule reaction with the transfer of a proton from one of the hydroxy groups of the molecule (Scheme). In all the CI(−) spectra there is a medium-intensity peak of the $(M - H - H_2)^-$ ion the origin of which can be explained by the dehydrogenation of the $(M - H)^-$ ion. In addition to this, in the CI(−) spectra there are two groups of peaks differing from the molecular mass by 15-19 and 30-36 a.m.u. The qualitative and quantitative features of these groups of ion peaks will be considered in an analysis of the spectra of the individual groups of alkaloids.

In the CI(−) spectra of the 8-monools (**1, 2**), the peak of the $[M - H]^-$ ion is the maximum peak, and there are no peaks of $(M - 15)^-$ and $(M - H_2O)^{-\cdot}$ ions. Each spectrum contains a doublet of the peaks of $(M - OH)^-$ and $M - H - H_2O)^-$ ions, the relative intensity of the latter being higher for the 1-hydroxy compound (**1**), which shows the partial involvement of the 1-OH group (Table 2). A triplet of peaks of the $(M - 32)^{-\cdot} - (M - 34)^{-\cdot}$ ions with a maximum on the $(M - 33)^-$ peak also stands out in the spectra. The formation of these ions takes place through the elimination of CH_3OH from the $M^{-\cdot}$, $(M - H)^-$, and $(M - H_2)^{-\cdot}$ ions, respectively. One of the possible variants of the formation of the $(M - 33)^-$ ions is shown in the scheme.

Because of the occurrence of ion-molecule reactions in the spectra of the compounds of the first group, and also in the spectra of many other alkaloids, weak peaks of $(M + 32)^{-\cdot}$ ions arising on the addition of methanol to $M^{-\cdot}$ are present.

The CI(−) of the 6-OMe-7,8-glycols (**3-8, 20**) depends greatly on the nature of the substituent at C-1. In the spectra of the 1-hydroxy- (**3, 4**) and 1-acetoxy- (**8, 20**) bases the maximum peak is that of the $(M - H)^-$ ion (Table 2). In the case of compounds (**3** and **4**) the peak of the $(M - H - H_2)^-$ ion has increased. With a decrease in the number of hydroxy groups in the molecule the intensity of this peak also falls. The distribution of the peaks of the $(M - 15)^- - (M - 19)^-$ ions is monotypical for this subgroup: the peak of the $(M - OH)^-$ group appears while the peaks of the $(M - H_2O)^{-\cdot}$ and $(M - H - H_2O)^-$ ions are weaker.

In all the spectra of the compounds of the second group the peak of $(M - 34)^{-\cdot}$ ion had a high intensity; for (**3**) and (**4**) it was the second strongest, while for (**5-7**) it was the 100% peak. The probable mechanism of its formation is shown in the scheme: the anion-radical $O^{-\cdot}$ abstracts protons from $M^{-\cdot}$, leaving in a form of an H_2O molecule, and the subsequent elimination of methanol gives $(M - 34)^{-\cdot}$ ions. The main source of the latter is the 6-OMe group. For compounds (**5-7**), an additional contribution to the formation of the $(M - 34)^{-\cdot}$ ions is made by the 1-O-Me group, although, judging from the intensities of the peaks of the $(M - R_1OH)^{-\cdot}$ and $(M - H_2 - R_1OH)^-$ ions (**3, 4, 8, 20**, Table 2), the mobility of the 1-OR_1 substituent is low.

The peaks of the $(M - 36)^{-\cdot}$ ions in the CI(−) spectra of (**3-7** and **20**) are fairly intense, the sequence of their formation probably being as follows: $(M - H)^- \rightarrow (M - H - H_2O)^- \rightarrow (M - 36)^{-\cdot}$. In addition to the peaks described above, in the spectra of the 6-OMe,7,8-diols attention is attracted by the peaks of the odd-electron fragments with MMs smaller by 48, 50, 52, 62, 64, 66, 78, and 80 a.m.u. They may originate by various alternative routes.

The peaks of the deprotonated molecular ions are the maximum peaks in the CI(−) spectra of the 3,4-epoxy- bases (**9, 10, 21**, Table 2). Within the subgroup the spectra are highly monotypical. A characteristic feature of these spectra is the presence of a triplet of the $(M - 17)^-$, $(M - 18)^{-\cdot}$, and $(M - 19)^-$ ions with a well-defined maximum on the $(M - 18)^{-\cdot}$ peak probably owing to the presence of the epoxy group. Another characteristic feature of the spectra of this group is the appearance of the peaks of the $(M - 30)^{-\cdot}$ $[(M - CH_2O)^{-\cdot}]$ peaks.

Among the cluster ions there is an ion with a mass 15 a.m.u. greater than the molecular mass which is due to the addition of a CH_4 molecule to the $(M - H)^-$ ion (**11-15, 17**).

The CI(−) spectra of alkaloids with a 7,8-MDOG form systems depending extremely sensitively on a change in the substituent at C-6. The distribution of the intensities of the peaks of the ions in the molecular region is uncharacteristic: in addition to the deprotonated molecular ion, of medium intensity, in the spectra of (**13**), (**15**) and (**17**) there is the 2.5-times weaker peak of the $(M - H - H_2)^-$ ion, while for (**15**) in this region the strongest peak was that of the $(M - H_2)^{-\cdot}$ ion (Table 2).

The peaks of ions differing from the MM by 15-19 a.m.u. are of medium intensity. Each compound had an individual pattern in this region. Let us dwell further on the nature of the ions giving the maximum peak in the CI(−) spectra of the alkaloids of the fourth group. The substituents at C-6 and the 7,8-MDOG probably participate in its formation. For the 6-dehydro derivatives (**12, 13**) the 100% peak is that of the $(M - 30)^{-\cdot}$ ion. The unstable molecular ion, eliminating a CH_2O molecule, is stabilized in a resonance-delocalized form (Scheme). In the spectra of deoxydelcorine (**11**) and 6-O-OMe-eldelidine (**17**) the maximum peaks are those of the $(M - 31)^-$ ion. Its origin is the loss of CH_2O from the 7,8-MDOG and of an H atom from C-6. Characteristic for the 6-hydroxy derivatives (**14, 15**) is the presence of the $(M - 32)^{-\cdot}$ ion as the 100% peak. In addition to the substituents mentioned above, the hydrogen of the C_6-OH group participates in its appearance. In the CI(−) spectra of the alkaloids of the fourth group there are, likewise, intense peaks of the $(M - 46)^{-\cdot}$, $(M - 48)^{-\cdot}$, $(M - 60)^{-\cdot}$, and $(M - 62)^{-\cdot}$ ions, but we shall not consider the mechanism of their formation, since the participation of various alternative substituents in this process is possible.

Thus, the CI(−) spectra of the diterpene alkaloids are characteristic only within narrow subgroups of compounds.

EXPERIMENTAL

The CI(+) and (−) spectra were obtained on a MS 25 RF (KRATOS) instrument. Combined CI/EI ion source, accelerating voltage 4 kV, ionization voltage 70 V, collector-stabilizing regime, emission current 50 μA, source temperature 250°C, temperature of the sample injection system 150-200°C.

The reagent gas for CI(+) was CH_4, and for CI(−) it was CH_4:N_2O (1:1), the pressure in the ionization zone being 0.1 mm Hg.

REFERENCES

1. Ya. V. Rashkes and M. S. Yunusov, Khim. Prir. Soedin., 481 (1984).
2, E. G. Sirotenko, Ya. V. Rashkes, V. A. Tel'nov, and M. S. Yunusov, Khim. Prir. Soedin., 95 (1989).
3. E. G. Sirotenko, Ya. V. Rashkes, A. S. Narzullaev, M. S. Yunusov, V. M. Matveev, and S. S. Sabirov, Khim. Prir. Soedin., 389 (1987).
4. M. S. Yunusov, Ya. V. Rashkes, B. T. Salimov, É. F. Ametova, and G. V. Fridlyanskii, Khim. Prir. Soedin., 525 (1985).
5. Ya. V. Rashkes, E. G. Mil'grom, and Yu. M. Mil'grom, Khim. Prir. Soedin., 384 (1993).
6. R. A. W. Johnstone, Mass Spectrometry for Organic Chemists, Cambridge University Press (1972) [Russian translation, Mir, Moscow (1975), p. 67].
7. K. L. Busch, A. Norstroem, M. M. Bursey, J. R. Hass, and C. A. Nilsson, Biomed. Mass Spectrom., **6**, 157 (1962).
8. L. R. Hogge and D. J. H. Olson, J. Chromatogr. Sci., **20**, 109 (1982).