MINOR ALKALOIDS IN COMMERCIAL SAMPLES OF COLCHICINE

Maria A. Iorio, Anna Mazzeo-Farina, Guido Cavina and Luigi Boniforti

Laboratory of Pharmaceutical Chemistry, Istituto Superiore di Sanità, 00161 Rome, Italy

Arnold Brossi

Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, MD 20014, USA

Abstract- Commercial samples of colchicine contain a total of about 5% of minor alkaloids as revealed by HPLC. Three minor alkaloids were isolated by preparative ThLC and identified by Mass Spectrometry to be N-formyldeacetylcolchicine, C-17-hydroxycolchicine and C-5(or 6)-hydroxycolchicine.

Examination by TLC of commercial samples of colchicine from different sources (Aldrich or Fluka Chemical Co.) revealed the presence of three faded spots running slower than that of colchicine. In order to determine if these "trace" compounds could be minor alkaloids of colchicine family, we isolated them by preparative ThLC. All operations were carried out in a dim light so as to avoid photochemical rearrangement of colchicine to β-lumicolchicine.[1]

Colchicine (1 g) was crystallized twice from hot ethyl acetate, each time decanting the mother liquor from the solid which separated after overnight standing. The combined mother liquors, after concentration to half-volume and separation of an additional crop of colchicine, resulted enriched by the three minor alkaloids. In Fig. 1 a HPLC chromatogram is shown with the original content of these "trace" alkaloids in colchicine commercial samples and in Table 1 some R_f values of colchicine and minor alkaloids are listed. In the conditions used for the chromatogram of Fig. 1, alkaloid 1 was not eluted; using a more polar solvent, 10% MeOH in $CHCl_3$ (isocratic), 1 showed a retention time of 8.1 min. Correspondence of the peaks on HPLC with spots on TLC (visualized with UV light, 254 nm), was obtained either by injecting samples of isolated minor alkaloids or by adding the same, sepa-

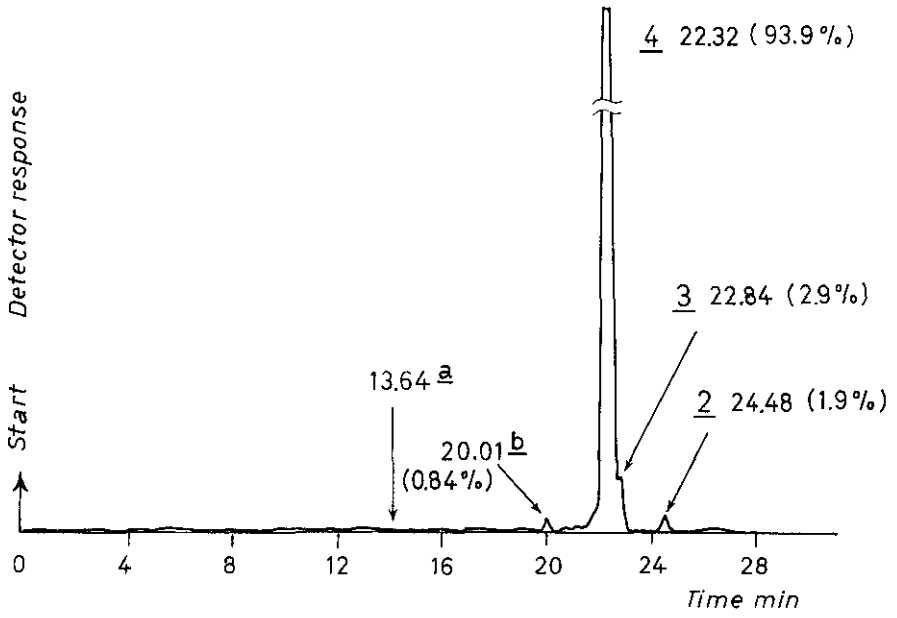


Fig.1- HPLC separation of minor alkaloids in commercial samples of colchicine (10 µg in 50 µl of $CHCl_3$). Retention time and % areas are listed. Hewlett-Packard 1084; column Lichrosorb Si-100 (Merck) 10 µ ; 25 cm x 4.5 mm i.d.; mobile phase: (A) H_2O-saturated $CHCl_3$ (prewashed with H_2O to remove EtOH); (B) same $CHCl_3$ containing 5% MeOH + 0.2-0.3% H_2O for complete saturation with H_2O; gradient elution starting from (A) with 10% (B) to 100% (B) in 20 min, followed by isocratic for 10 min and reequilibration to the starting condition in 20 min before further analysis; room temperature; flow rate 1 ml/min; UV detector at 254 nm.
a Peak visible only in samples stored in day-light and attributed to β-lumicolchicine by comparison to an authentic sample of β-lumicolchicine.
b this peak is due to an unidentified impurity.

rately, to the total colchicine and verifying the increased intensity of the corresponding peak. All the minor alkaloids were isolated from the mother liquor by preparative ThLC (SiO_2 F 254, layer thickness 2 mm, 20 x 20 cm, developing system, solvent I or II, positive bands scraped off, transferred to glass filter tubes and eluted with $CHCl_3$-MeOH 1:1).

The chemical structure of the three minor alkaloids was elucidated by mass spectrometry (LKB spectrometer equipped with a Digital PDP 11 data processing system;

Table 1- R_f of colchicine and minor alkaloids [a].

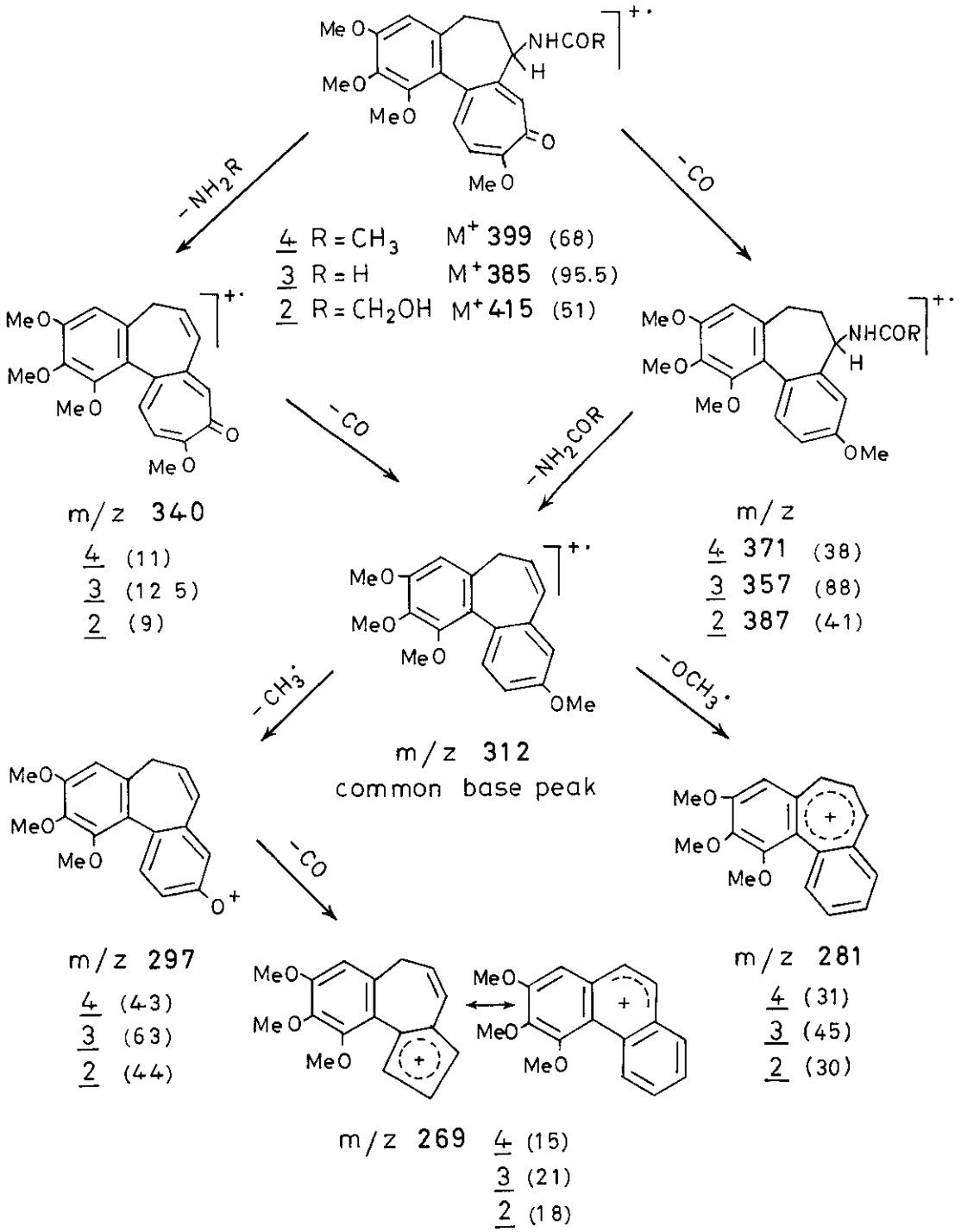
Compd	I	II	III
1 [b]	0.23	0.07	0.08
2	0.49	0.26	0.08
3	0.55	0.35	0.30
4 (colchicine)	0.60	0.48	0.38
β-lumicolchicine [c]	0.70	0.70	0.69

I. Stratocrom(Carlo Erba) SI F254, 5 x 20 cm; $CHCl_3$-MeOH-NH_4OH (90:9:1).
II. Same plates; $CHCl_3$-Me_2CO-Et_2NH (50-40-10).
III. Stratocrom AL F254; 2.5% MeOH in $CHCl_3$.
[a] A small spot at the starting point in the three solvent systems was visible if mother liquor was applied to the plates. It corresponded to colchiceine by comparison with TLC behaviour of an authentic sample.
[b] From the size of the spot the concentration of 1 approximated 1/2 that of 2 (see Fig.1).
[c] This spot appeared only in old solutions stored in day-light.

samples applied by direct inlet and probe usually heated from 25-200°).

The fragmentation pattern of 3 and 2 upon electron impact showed a significant similarity to that of colchicine, indicating that differences in structure were located only in the C-7-acylamide side-chain (Scheme 1). Colchicine mass spectrum and its interpretation has been previously reported [2]. After electron impact either of colchicine or of 3 and 2 to provide the molecular ion, fragmentation proceeds in two directions, both leading to the base peak at m/z 312. Further fragmentation pattern below m/z 312 is common to all the three compounds except for the very low mass range of the spectrum characterized by an intense peak at m/z 43 for colchicine and m/z 31 for 2; peaks of no particular intensity were visible in the same region for 3. It is deduced from its spectral fragmentation that 3; M^+ 385, is the well-known alkaloid N-formyl-deacetylcolchicine[3a,b], whose mass spectrum has been previously reported [2]. Mass fragmentation of alkaloid 2 of molecular ion 415 showed that it possessed a C-7-$NHCOCH_2OH$ chain instead of a C-7-$NHCOCH_3$ one as in colchicine. The presence of a fragment at m/z 31 (36.5%) instead of one at m/z 43 ($COCH_3$, 73%) as in colchicine, indicated the presence of a CH_2OH group in the side-chain (Fig.2 and Scheme 1). This ion is characteristic of alcohols and it is due to the cleavage of the carbon-carbon bond next to the heteroatom [4].

Scheme 1. Fragmentation pattern of colchicine (4) and minor alkaloids 3 and 2: m/z (relative intensity in %).



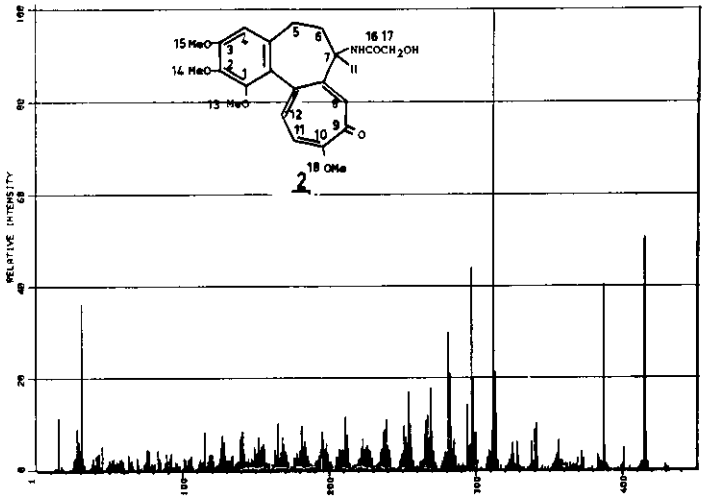


Fig. 2. Mass spectrum of alkaloid 2

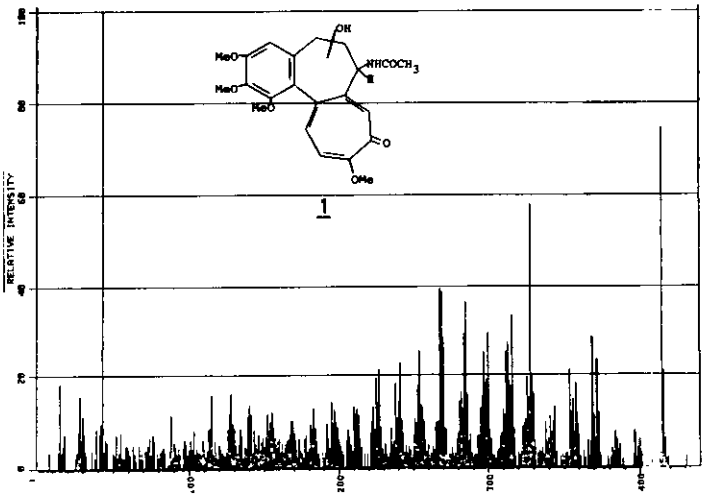


Fig. 3. Mass spectrum of alkaloid 1.

Ketoalcohols of the type RCHOHCOR' also exhibit intense peaks due to $RCHOH^+$ and COR'^+ ions. Further support to this interpretation came from the deuterium exchange experiment. The direct inlet of a solution of 2 in $CDCl_3$, shaken with a drop of D_2O, caused, besides m/z 31, the appearance of a new peak at m/z 32 (31-32 relative intensity: 40/24) as expected if an exchangeable hydroxyl hydrogen was present in this fragment. Either the molecular ion 415 or the fragment ion 387 (M^+ -CO) were flanked by two new peaks shifted by one or two mass units, due to the partial exchange either of the hydroxyl or amide hydrogen (relative intensity , m/z 415-416-417 : 20.5/30.5/19 and m/z 387-388-389 : 18.7/25.4/14.3). Thus, alkaloid 2 is C-17-Hydroxycolchicine, m.p. 131-133 °dec. (from ethyl acetate) which to our knowledge, has not yet been isolated from Colchicum plants, even though Šantavý et al.[5] have recently identified in the leaves of Colchicum autumnale a new alkaloid, 2-demethylcolchifoline (isolated as diacetates) with a hydroxyacetyl chain at C-7. In this respect, alkaloid 2 could be named colchifoline.

The more polar alkaloid 1 showed the same mass ion (M^+ 415) as 2, but different fragmentation pattern (Fig.3). Its molecular ion fragmented by three major processes: a loss of H_2O and CO, m/z 369 (24%); b) loss of NH_2COMe and CO, m/z 328 (60.6%) and c) loss of COMe and CO, m/z 344 (13%). Each one of these ions underwent stepwise fragmentation leading to similar ions shifted of 1-3 mass units. This alkaloid appeared to be hydroxylated in ring B and could be represented either as a C-5 or a C-6-hydroxycolchicine. Its m.p. 169-171 °dec. (from ethyl acetate corresponds to that reported for β-6-hydroxycolchicine (colchiciline) isolated from C. latifolium [6], thus, alkaloid 1 could possibly be identical with this alkaloid.

References

1. R.Grewe and W.Wulf, Chem.Ber., 1951, 84, 621.
2. J.M.Wilson, M.Ohashi, H.Budzikiewicz, F.Šantavý and C.Djerassi, Tetrahedron, 1963, 19, 2225.
3a. F.Šantavý and T.Reichstein, Helv.Chim.Acta, 1950, 33, 1606; 3b. R.F.Raffauf, A.L.Farren and G.E.Ullyot, J.Amer.Chem.Soc. 1953, 75, 3854 isolated 3 from U.S.P.colchicine.
4. H.Budzikiewicz, C.Djerassi and D.H.Williams, "Mass Spectrometry of Organic Compounds", Holden-Day Inc., San Francisco, 1967, pp.94-103.
5. P.Sedmera, H.Potěšilová, V.Malichová, V.Preininger and F.Šantavý, Heterocycles, 1979, 12, 337.
6. H.Potěšilová, L.Dolejš, P.Sedmera and F.Šantavý, Collect.Czech.Chem.Comm., 1977, 42, 1571.

Received, 23rd February, 1980