ISOLATION AND IDENTIFICATION OF ALKALOIDS FROM SEEDS OF *Colchicum latifolium* s. s.*

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In the seeds of *Colchicum latifolium* s. s. from Yugoslavia (Macedonia), thirteen neutral and phenolic alkaloids and six alkaloids of basic nature have been detected. The newly isolated alkaloids are 10,11-epoxycolchicine $C_{22}H_{25}NO_7$, colchiciline $C_{22}H_{25}NO_7$, 3-demethyl-N-formyl-N-deacetylcolchicine, 2,3-didemethylcolchicine, autumnaline and some other alkaloids in traces (alkaloids CL-1, CL-2, and, probably, 2-demethylcolchiciline). Furthermore, the UV spectroscopic differentiation between 3-demethyl- and 2-demethylcolchicine and their derivatives in alkaline medium has been described.

From the seeds of *Colchicum autumnale* L. growing in Central Europe there were isolated[1-4] or detected by paper chromatography and thin-layer chromatography on silica gel[5] the substances given in the Tables I and II. Many of those substances were also isolated by Bellet and coworkers[6-9] from *Colchicum* seeds collected in Yugoslavia (the species has, however, not been precisely defined). Furthermore, they found the colchicoside (an alkaloid-glycoside) and the substance $C_{22}H_{25}NO_4S_2$, the structure of which has not been resolved as yet.

A preliminary analysis of the commercially obtained seeds from Yugoslavia showed that the spectrum of the alkaloids contained therein differed from that of the seeds of *C. autumnale* indigenous to Central Europe. Therefore we tried to establish the origin and the plant species of the supplied *Colchicum* seeds whose size and coloration were identical with those of the seeds from *C. autumnale*[10]. We found that the seeds were collected in Macedonia where the only *Colchicum* plant[11] flowering in autumn and yielding seeds in spring is *C. latifolium* s. s. In this paper, the results of the qualitative and quantitative representation of alkaloids in the seeds of this plant have also been compared with those obtained earlier[2,3,5] from the seeds of *C. autumnale*. In an other paper[12], we have studied the alkaloids contained in the corms, flowers and flower stalks of *C. latifolium* collected in Macedonia. From those parts of the plant there have been isolated or identified similar alkaloids as those described in this communication.

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In the seeds of *C. latifolium*, we demonstrated a total of 13 substances of neutral--phenolic and six substances of basic nature (Tables I and II). We did not succeed

TABLE I

Content of Alkaloids (%) in the Neutral-Phenolic Portion from the Seeds of *C. latifolium* and *C. autumnale* and Their hR_F in the Systems S_1 and S_3

Compounds	S_1	S_3	*C. latifolium* and *C. autumnale*[a]
$C_{22}H_{25}O_4NS_2$	—	—	0·0033 (9)
β-Lumicolchicine	82	—	0·0008 0·007 (2) 0·006 (6)
γ-Lumicolchicine	70	—	traces
10,11-Epoxycolchicine	68	81	0·0009
Colchicine	56	74	0·50 0·98 (2) 0·55 (3) 0·935 (6)
N-Formyl-N-deacetylcolchicine	44	63	0·005 0·032 (2) 0·15 (3) 0·022 (6)
Alkaloid CL-1	38	59	traces
2-Demethylcolchicine	32	52	traces
3-Demethyl-N-formyl-N-deacetylcolchicine[b]	25	38	0·0002
Colchiciline	23	36	0·011
Alkaloid CL-2	20	32	traces
3-Demethylcolchicine	18	29	traces 0·046 (2) 0·05 (3) 0·048 (6)
2-Demethylcolchiciline	—	21	traces
2,3-Didemethylcolchicine[c]	start	18	traces
Colchicoside	start	7	(6)

[a] References for the seeds *C. autumnale* are given in parentheses. [b] This substance was demonstrated by Canonica and coworkers[18] in the corms of *Gloriosa superba*. In the paper[19] on p. 207 the substance was confused with 2-demethyl-N-formyl-N-deacetylcolchicine (old numeration). [c] The presence and the constitution of this alkaloid were assumed on the basis of the ^{1}H-NMR spectra.

TABLE II

Content of Alkaloids (%) in the Basic Portion From the Seeds of *C. latifolium* and *C. autumnale* and Their hR_F Values in the Systems S_2 and S_3

Compounds	S_2	S_3	*C. latifolium* and *C. autumnale*[a]
β-Lumidemecolcine	78	—	traces
γ-Lumidemecolcine	70	—	traces
Demecolcine	56	76	0·0011 0·003 (2) 0·014 (3)
Autumnaline	48	—	traces
2-Demethyldemecolcine	32	67	0·015 0·014 (3, 8)
3-Demethyldemecolcine	22	41	traces

[a] References for the seeds *C. autumnale* are given in parentheses.

to isolate the colchicoside and the substance $C_{22}H_{25}NO_3S_2$ which Bellet and Muller[6,9] had isolated in good yield. The newly found alkaloids were 10,11-epoxycolchicine[13], colchiciline[13], 3-demethyl-N-formyl-N-deacetylcolchicine, γ-lumicolchicine, 2-demethylcolchicine, 2,3-didemethylcolchicine, 3-demethyldemecolcine, and β- and γ-lumidemecolcine. Traces of amorphous tropolone alkaloids CL-1, CL-2 and, probably, of 2-demethylcolchiciline were found. The presence of 2-demethylcolchiciline is assumed on the basis of its colour reaction with iodoplatinate. In this material we also found autumnaline which had already earlier been detected[14,15] in *C. byzanthinum* and then isolated[16] from *C. cornigerum*. 3-Demethyldemecolcine was so far isolated only from the corms of *C. cornigerum*[16]. From *C. latifolium*, we obtained new noncrystalline substances of tropolone nature named CL-1 and CL-2. The seeds also showed to contain a small quantity of an amorphous substance, probably 2-demethylcolchiciline.

The content of colchicine in dry seeds of *C. autumnale*[2,3,5,10,17] ranges between 0·35 and 0·98%. In seeds of *C. latifolium*, the content of colchicine was found to be 0·5%. This material is just as suitable for an industrial work-up as the seeds of *C. autumnale*. In *C. latifolium*, the quantity of N-formyl-N-deacetylcolchicine and demecolcine is smaller than that contained in *C. autumnale* (Tables I and II).

While carrying out the identification of tropolone alkaloids with a phenolic group

at $C_{(2)}$ or $C_{(3)}$ (Table III),* we noticed that their UV spectra in alkaline–ethanolic medium differed from those in neutral ethanolic medium and, therefore, we undertook to study this problem in more detail. The above-mentioned isomers can also be differentiated by IR and ^{1}H-NMR spectroscopy[18,23,24] but, on using the latter method, the signals of the methoxyl groups at $C_{(2)}$, $C_{(3)}$ and $C_{(10)}$ are almost overlapping. Furthermore, the isomers with a hydroxyl group at $C_{(2)}$ show higher[5,19] hR_F values.

The UV spectra of all these compounds are identical in neutral and acidic media. A considerable difference is, however, seen in alkaline medium where the compounds with a hydroxyl group at $C_{(3)}$ yield a mesomeric anion in the structure of which participates significantly the quinoid canonic structure. Consequently in alkaline medium, the UV spectra differ greatly from those of the non-ionized compound. On the contrary, in compounds with a hydroxyl group at $C_{(2)}$ the anion cannot be affected by such a mesomerism. Consistent with this finding is the insignificant difference between the UV spectra of the $C_{(2)}$ hydroxy derivatives in neutral and basic solutions (Fig. 2).

In alkaline medium, the bands *A* and *B* of substances *Ib* and *Vb* are split into two bands (A^1, A^2 and B^1, B^2) with the A^1 band bathochromically shifted (Fig. 1). In the

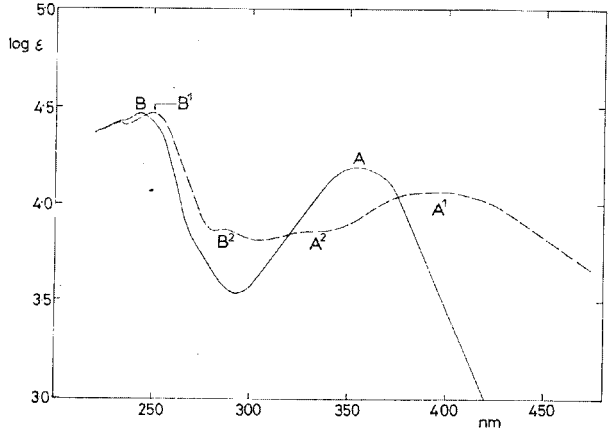

FIG. 1

UV Spectra of 3-Demethylcolchicine (*I*) ——— in Ethanol, ----- in Ethanolic NaOH

* In this Table, the numbering of the carbon atoms is that according to Wildman[20,21], whereas in previous papers of this series, the reversed numbering is used; the latter numbering was also employed by Boit[22] and by Ricca and Danielli[23]. The numbering introduced by Wildman corresponds to the IUPAC nomenclature and, therefore, it will also be used by us from now on.

TABLE III

Colchicine Alkaloids With a Phenolic Group and Some of Their Properties

Compound	M.p., °C $[\alpha]_D$ in $CHCl_3$	hR_F value	UV spectra	
			ethanol	ethanol–0·1M-NaOH
3-Demethylcolchicine[22,23,31] (*I*) (Substance C)	190 −130°	29	max.: 243 nm (log ε 4·46), 355 (4·19); min.: 232 (4·41) sh, 293 (3·54)	max.: 251 nm (log ε 4·46), 286 (3·86), 395 (4·06); min.: 232 (4·40), 280 (3·84), 305 (3·80), 340 (3·86) sh
2-Demethylcolchicine[22,23,31] (*II*) (Substance E_1)	180 −133°	52	max.: 243 nm (log ε 4·46), 355 (4·20); min.: 222 (4·38), 292 (3·55)	max.: 247 nm (log ε 4·45), 290 (3·86)sh, 356 (4·19); min.: 280 (3·85); 309 (3·70)
3-Demethyl-N-formyl-N-deacetylcolchicine[19] (*III*)	267 —	33	max.: 232 nm (log ε 4·48), 245 (4·44)sh, 357 (4·19); min.: 293 (3·58)	max.: 225 nm (log ε 4·52), 245 (4·51), 279 (3·99), 374 (4·00); min.: 273 (4·00), 307 (3·70)
2-Demethyl-N-formyl-N-deacetylcolchicine[18,19] (*IV*)	[a] —	56	max.: 243 nm (log ε 4·46), 355 (4·20); min.: 232 (4·41) sh, 293 (3·54)	max.: 247 nm (log ε 4·45), 290 (3·86), 356 (4·19); min.: 280 (3·85), 309 (3·70)
3-Demethyl-N-methyl-N-deacetylcolchicine[32] (*V*) (Substance CC-7)	222 −128°	41	max.: 244 nm (log ε 4·47), 356 (4·20); min.: 292 (3·65)	max: 235 nm (log ε 4·44), 250 (4·47), 291 (3·87), 318 (3·82) sh, 394 (4·01); min.: 239 (4·44), 283 (3·87), 305 (3·84), 338 (3·76)
2-Demethyl-N-methyl-N-deacetylcolchicine[3,8] (*VI*) (Substance S)	138 −119°	67	max.: 241 nm (log ε 4·49), 355 (4·19); min.: 290 (3·61)	max.: 244 nm (log ε 4·60), 287 (3·96), 355 (4·13); min.: 278 (3·94), 309 (3·80)

2,3-Didemethyl-N-deacetylcolchicine[19] (*VII*)	[a] —	20	max.: 245 nm, 355 (log ε 4·15); min.: 221, 236, 294 (log ε 3·60)	max.: 240 nm, 258, 297, 356 sh, 405; min.: 244, 289, 313
3-Demethylcolchicine glucoside[6,7] (*VIII*)	218 —360°[b]	7	max.: 244 nm, 345; min.: 290	
Colchiceine[18,28] (*IX*)	179 —253°	8	max.: 243 nm (log ε 4·47), 352 (4·24); min.: 216 (4·42), 295 (3·64)	max.: 241 nm (log ε 4·49), 259 (4·40) sh, 352 (4·24), 367 (4·29) sh, 403 (4·04); min.: 214 (4·33), 253 (4·41), 307 (3·22), 362 (4·29), 398 (4·03)
3-Demethylcolchiceine[19] (*X*)[c]	— —	0	—	max.: 242 nm (log ε 4·71), 258 (4·64) sh, 266 (4·49) sh, 302 (3·94), 368 (4·46), 397 (4·45); min.: 224 (4·53), 294 (3·94), 312 (3·92), 383 (4·45)
2-Demethylcolchiceine[19] (*XI*)[c]	— —	0	—	max.: 236 nm (log ε 4·78), 280 (4·10) sh, 343 (4·49), 380 (4·35); min.: 214 (4·50), 301 (3·94), 366 (4·39) sh
3-Demethyl-N-formyl-N-deacetyl-colchiceine[19] (*XII*)[c]	— —	0	—	max.: 242 nm (log ε 4·70), 265 (4·35) sh, 365 (4·31), 397 (4·32); min.: 220 (4·51), 304 (3·46), 379 (4·31)

[a] Amorphous; [b] in water; [c] prepared for the measurements from the weighed quantity of the initial substance.

same medium the band *A* of the substance *IIIb* is bathochromically shifted to c. 390 nm) but it remains single and only the band *B* is split (Table III). A similar manifestation of the quinoid canonic structure was also observed[25,26] in protoberberine bases with a phenolic group at $C_{(3)}$, and in some aporphine alkaloids[27,28]. Thus the differentiation between colchicine isomers with a phenolic group at $C_{(2)}$ or at $C_{(3)}$ is facilitated. Splitting of the band *B* in alkaline-ethanolic medium into the bands B^1 and B^2 is also observed in 2-demethylcolchicine derivatives (*II*, *IV*, *VI*) where, how-

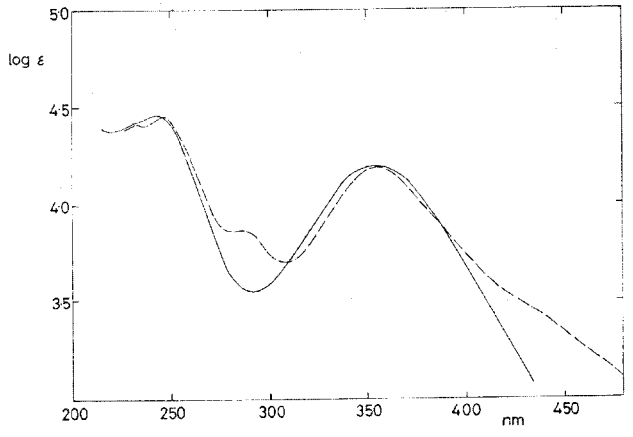

FIG. 2

UV Spectra of 2-Demethylcolchicine (*II*) ——— in Ethanol, - - - - - in Ethanolic NaOH

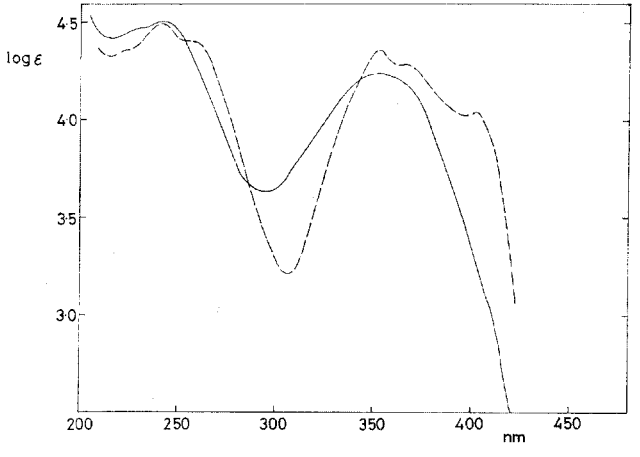

FIG. 3

UV Spectra of Colchiceine (*IX*) ——— in Ethanol, - - - - - in Ethanolic NaOH

ever, the band B^2 is far more marked with a maximum at c. 290 nm, whereas in the compounds *Ib*, *IIIb* and *Vb* the maximum is at c. 280 nm (Figs 1 and 2).

Colchiceine (*IX*) may exist[24,29] in two tautomeric forms in which the keto group of the tropolone ring is either at $C_{(9)}$ (normal series) or at $C_{(10)}$ (iso-series). In alkaline medium, the UV spectrum of colchiceine (*IX*) is given by the mesomerism of the corresponding anion (Fig. 3). We studied the spectra in alkaline-ethanolic medium of compounds *X*–*XII* with a free phenolic group at $C_{(2)}$ or at $C_{(3)}$ or with a saponified methoxyl group at $C_{(10)}$ which correspond to colchiceine (*IX*). These two types of compounds *X* and *XI* exhibit analogous UV spectra; the two long wavelength bands of 3-demethylcolchiceine (*X*) and of 3-demethyl-N-formyl-N-deacetylcolchiceine (*XII*) are shifted more bathochromically than those of 2-demethylcolchiceine (*XI*) (Fig. 4). Hence, in this manner, it is also possible to differentiate the isomers with a phenolic group at $C_{(2)}$ or at $C_{(3)}$ if we prepare the corresponding colchiceine derivative which is then examined by UV spectroscopy in neutral and alkaline media.

EXPERIMENTAL

The melting points were measured on a Kofler block and are not corrected. The isolation of the substances and their separation by column chromatography on Al_2O_3 was carried out as described[1–3]. The elemental composition[30] was determined by mass spectrometry MS-9 (England) with double focussing. The IR spectra were measured in chloroform and in KBr discs on a Perkin-Elmer or on a UR-10 Zeiss, Jena instrument, the ^{1}H-NMR spectra on a Varian T-60 (60 MHZ), and the UV spectra on a Unicam SP. 700, spectral slit width 0·45 nm at 230 nm, 0·65 at 300 nm, 0·8 nm at 400 nm, and 0·9 nm at 500 nm. The substance (more than 0·5 mg, weighed with a precision of 0·01 mg) was dissolved in 95% of ethanol to concentration of 10^{-4}M. The

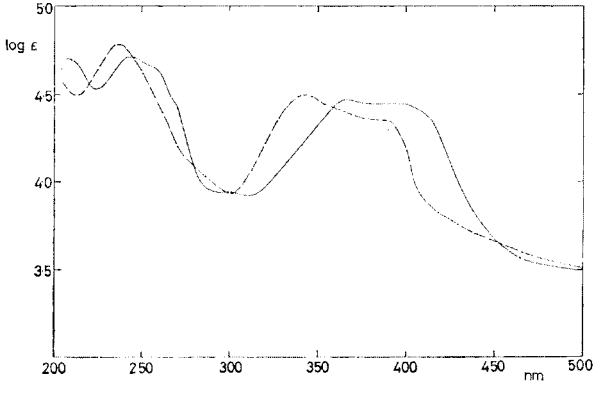

FIG. 4

UV Spectra in Ethanolic Sodium Hydroxide
3-Demethylcolchiceine (*X*) ———, 2-Demethylcolchiceine (*XI*) · · · · · .

ethanolic solution (2·5 ml) of some of the compounds was made alkaline with 0·1M-NaOH (0·4 ml). The solutions were measured in 1·00 cm silica cells; for higher values than 17000, cells of 0·20 cm length were used or the solution was diluted to a concentration of 10^{-5}M and measured in 1·00 cm cells. Thin-layer chromatography[5] was carried out on silica gel G using the solvent systems benzene–ethyl acetate–diethylamine (50 : 40 : 10) + 4% of methanol (S_1), benzene–ethyl acetate–diethylamine (70 + 20 + 10) (S_2), and chloroform–acetone–diethylamine (50 : 40 : 10) + 8% methanol (S_3). Detection under UV light, with Dragendorff reagent or with potassium iodoplatinate.

Extraction: Dry seeds (27 kg) of *C. latifolium* s. s. from Yugoslavia (Macedonia, collected in 1971) were ground and extracted in two batches (13 and 14 kg) with a total of 200 l of methanol. After concentration, the residue (6 liters) was extracted with ether (25 l). The aqueous residue was acidified with acetic acid to pH 4·5—5·0 and taken up into chloroform (15 l). Yield 201·0 g (neutral-phenolic chloroform extract). The aqueous portion was made alkaline with ammonia and taken up again into chloroform (16 l). Yield 12·73 g (basic chloroform extract). The aqueous extract was then cooled, made neutral with hydrochloric acid, and shaken with a mixture of chloroform–ethanol 4 : 1 (6 l); however, we did not succeed to obtain any substance. The neutral-chloroform extract yielded crystalline colchicine (63·0 g). The mother liquors were separated by column chromatography on Al_2O_3 and by thin-layer chromatography on silica gel. The basic chloroform extract gave crystalline 2-demethyldemecolcine (2·5 g). The mother liquors were also subjected to column chromatography on Al_2O_3.

Separation of the neutral-phenolic chloroform extract: Column chromatography on Al_2O_3 gave 0·21 g of β-lumicolchicine (solvent system ether–chloroform 1 : 1); 60·1 g of colchicine (chloroform, chloroform–methanol 99 : 1), 1·12 g of N-formyl-N-deacetylcolchicine (chloroform–methaneol 98 : 2); 2·57 g of colchiciline (chloroform–methanol 96 : 4), and 0·05 g of 3-demethyl-N-formyl-N-deacetylcolchicine (chloroform–methanol 96 : 4). The mother liquors (18·1 g) after colchicine were rechromatographed on a column of Al_2O_3 to afford 0·23 g of 10,11-epoxycolchicine (ether–chloroform 1 : 1) and 2·1 g of colchicine. The total yield of colchicine was 125·3 g. Thin-layer chromatography (S_1) revealed the presence of γ-lumicolchicine, 2-demethylcolchicine, 3-demethylcolchicine, and a substance of a hR_F value 38. The amorphous residues of mother liquors from the individual fractions after column chromatography were combined according to their polarity into three portions: substances extractable with ether, ether–chloroform, and chloroform–methanol; the last two were subjected to preparative chromatography on a thin layer of silica gel (S_3). There was obtained a minute quantity of the substances CL-1 (*m/e* 415 $C_{22}H_{25}NO_7$, yielded the fragment *m/e* 356 $C_{20}H_{22}NO_5$), colchiciline, the tropolone alkaloid CL-2 (*m/e* 401, fragments 371, 357), 2-demethylcolchiciline and, finally, 2,3-didemethylcolchicine. On thin-layer chromatography, the substances of the colchiciline type gave intensive violet spots on spraying with iodoplatinate.

Separation of the basic chloroform extract: Column chromatography on Al_2O_3 yielded 0·29 g of demecolcine (chloroform–methanol 99 : 1) and 0·32 g of 2-demethyldemecolcine (chloroform–methanol 98 : 2). The presence of β-lumidemecolcine, autumnaline, and 3-demethyldemecolcine was detected by analytical and preparative thin-layer chromatography on silica gel (S_2).

Saponification of I—III *for UV measurements*: An accurately weighed substance (c. 1 mg) was dissolved in 4 ml of 0·1M-NaOH and heated on a boiling water bath for 2 h. After cooling, an equivalent amount of ethanol was added to obtain a concentration of 10^{-4}M. The thus prepared solution was then measured photometrically.

Newly Isolated Substances

10,11-*Epoxycolchicine*: The substance $C_{22}H_{25}NO_7$ was obtained by column chromatography of the mother liquors after colchicine. M.p. 251–253°C (ethyl acetate); $[\alpha]_D^{22}$ −237° ± 4° (*c* 1·68 in chloroform), hR_F 68 (S_1), copper-coloured spot in the UV light, with iodoplatinate beige, reaction with conc. sulphuric acid brown-red. For the structure see[13].

Colchiciline: The substance $C_{22}H_{25}NO_7$ was obtained in yellow crystals, m.p. 170–171°C (decomposition) (ethyl acetate); $[\alpha]_D^{22}$ −121° ± 4° (*c* 1·93 in chloroform); hR_F 23 (S_1), 33 (S_3), yellow spot in the UV light, after spraying with iodoplatinate violet. For the structure see[13].

3-*Demethyl*-N-*formyl*-N-*deacetylcolchicine*: The substance $C_{20}H_{21}NO_6$, m.p. 263–267°C (ethyl acetate); $[\alpha]_D^{22}$ −180° ± 5° (*c* 0·30 in chloroform), hR_F 36 (S_3), after spraying with iodoplatinate yellow, reaction with conc. sulphuric acid yellow. The structure of the substance was assigned on the basis of mass spectrometry[18], UV, IR, and NMR spectroscopy. The location of the phenolic group was inferred from the bathochromic shift of the band at 357 nm (shift to 374 nm) in alkaline-ethanolic medium.

2,3-*Didemethylcolchicine*: The substance did not crystallize and gave a yellow colour reaction with conc. sulphuric acid. Ultraviolet spectroscopy showed a band at 355 nm (log ε 4·15), minimum at 294 nm (log ε 3·60), which is indicative of a tropolone ring. This band is shifted bathochromically (405 nm) in alkaline–ethanolic medium, which shows (see[14]) the presence of a hydroxyl group at $C_{(3)}$. ^{1}H-NMR spectroscopy revealed only two methoxyl groups, namely at $C_{(1)}$ (218 c.p.s.) and at $C_{(10)}$ (241 c.p.s.)[19].

2-*Demethylcolchiciline*: This substance could not be obtained in a larger quantity and it remained amorphous. It is assumed, on the basis of the colour of the spots after detection in UV light (254 and 366 nm) and after spraying with different reagents[5] that it is a substance of colchiciline type with a tropolone ring. The substance does not exhibit a bathochromic shift in ethanolic-alkaline medium and the low hR_F value shows that it has a free phenolic group which is most probably located at $C_{(2)}$.

REFERENCES

1. Šantavý F.: Chem. Listy *42*, 177 (1948).
2. Šantavý F., Reichstein T.: Helv. Chim. Acta *33*, 1606 (1950).
3. Šantavý F., Talaš M.: This Journal *29*, 141 (1954).
4. Šantavý F.: This Journal *35*, 2857 (1970).
5. Potěšilová H., Hrbek H., jr, Šantavý F.: This Journal *32*, 141 (1967).
6. Bellet P.: Ann. Pharm. Fr. *10*, 81 (1952).
7. Bellet P., Amiard G., Pesez M., Petit A.: Ann. Pharm. Fr. *10*, 241 (1952).
8. Muller G., Bellet O.: Ann. Pharm. Fr. *13*, 81 (1955).
9. Bellet P., Muller G.: Ann. Pharm. Fr. *13*, 84 (1955).
10. Šantavý F., Buchníček J.: Pharm. Acta Helv. *24*, 20 (1949).
11. Stefanov B.: *Monographie der Gattung* Colchicum L., Vol. XXII. Akad. d. Wissenschaft, Sofia 1926.
12. Gašić O., Potěšilová H., Šantavý F.: Unpublished results.
13. Dolejš L., Potěšilová H., Sedmera P., Šantavý F.: Unpublished results.
14. Barker A. C., Battersby A. R., McDonald E., Ramage E., Clements J. H.: Chem. Commun. *1967*, 390.
15. Battersby A. R., Ramage R., Cameron A. F., Hannaway C., Šantavý F.: J. Chem. Soc. (C) *1971*, 3514.

16. Potěšilová H., Šantavý J., El-Hamidi A., Šantavý F.: This Journal *34*, 3540 (1969).
17. Buchníček J.: Pharm. Acta Helv. *25*, 389 (1950).
18. Canonica L., Danielli B., Manitto P., Russo G., Bombardelli E.: Chim. Ind. (Milan) *49*, 1304 (1967).
19. Thakur R. S., Potěšilová H., Šantavý F.: Planta Med. *28*, 201 (1975).
20. Wildman W. C. in the book: *The Alkaloids, Chemistry and Physiology* (R. H. F. Manske, Ed.), Vol. VI, p. 257;
21. Wildman W. C., Pursey B. A. in the book: *The Alkaloids, Chemistry and Physiology* (H. R. F. Manske, Ed.), Vol. XI, p. 407. Academic Press, New York 1960 and 1968.
22. Boit H.-G.: *Ergebnisse der Alkaloid-Chemie bis 1960*, p. 28. Akademie-Verlag, Berlin 1961.
23. Ricca G. S., Danielli B.: Gaz. Chim. Ital. *99*, 133 (1969).
24. Cross A. D., Hrbek J., jr, Kaul H. L., Šantavý F.: *Beiträge Biochem. Physiol. von Naturstoffen. Festschrift K. Mothes*, p. 97. Fischer, Jena 1965.
25. Cava M. P., Reed T. A., Beal J. L.: Lloydia *28*, 73 (1965).
26. Gašparec Z., Weber K.: Croat. Chem. Acta *39*, 175 (1967).
27. Preininger V., Hrbek J., jr, Samek Z., Šantavý F.: Acta Univ. Olomuc., Fac. Med. *52*, 5 (1969).
28. Preininger V., Hrbek J., jr, Samek Z., Šantavý F.: Arch. Pharm. (Weinheim) *302*, 808 (1969).
29. Šantavý F.: Publ. Fac. Med. Brno *19*, 149 (1946).
30. Wilson J. M., Ohashi M., Budzikiewicz H., Šantavý F., Djerassi C.: Tetrahedron *19*, 2225 (1963).
31. Šantavý F., Talaš M., Tělupilová O.: This Journal *18*, 710 (1953).
32. Saleh M., El-Gangihi S., El-Hamidi A., Šantavý F.: This Journal *28*, 3413 (1963).
33. Schönharting M., Pfaender P., Rieker A., Siebert G.: Hoppe-Seyler's Z. Physiol. Chem. *354*, 421 (1973).

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Note added in proof: Siebert and coworkers[33] found already earlier that 2- and 3-demethylcolchicine exhibit different UV spectra in neutral and alkaline medium but they did not study this effect in detail.