CONSTITUTION OF THREE ALKALOIDS (10,11-EPOXYCOLCHICINE, COLCHICILINE AND 3-DEMETHYL-N-DEACETYL-N-FORMYLCOLCHICINE) ISOLATED FROM THE SEEDS OF *Colchicum latifolium* S. S.*

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The structure elucidation of the drug components, 10,11-epoxycolchicine (*I*), colchiciline (6β-hydroxycolchicine (*III*)) and 3-demethyl-N-deacetyl-N-formylcolchicine (*VI*) were described.

From the seeds, corms, and flowers of *Colchicum autumnale* L., in addition to colchicine, some new alkaloids with tropolone ring and their lumiderivatives could be isolated[1,2] as well as the phenethyltetrahydroisoquinoline alkaloid autumnaline[3]. The seeds *C. autumnale* of Yugoslavian origin gave[4,5] the alkaloid-glycoside colchicoside and a substance $C_{22}H_{25}NO_4S_2$ of unknown structure[6].

From the seeds of *C. latifolium* S. S. of Yugoslavian origin (Macedonia), we isolated[7] two new alkaloids – epoxycolchicine (*I*) and colchiciline (*III*) – whose structures are being elucidated in this paper. There are also described the mass spectra of 3-demethyl-N-deacetyl-N-formylcolchicine (*VI*). The latter substance has also been isolated[7] from *C. latifolium*.

10,11-Epoxycolchicine (*I*) is ascribed the formula $C_{22}H_{25}NO_7$ (high resolution MS). It contains one oxygen atom more than colchicine and is therefore isomeric with the artificially prepared oxycolchicine[8-10] (*II*). In the mass spectrum of epoxycolchicine (Scheme 1), the base peak arises from the ionized molecule by the elimination of a molecule of acetamide. This mass spectrum differs from that of oxycolchicine (*II*). The M – CO ion is much less abundant than in colchicine and other alkaloids containing an intact tropolone system[11,12]. The ^{1}H-NMR spectrum provides evidence of the relationship of epoxycolchicine to colchicine. It exhibits singlets

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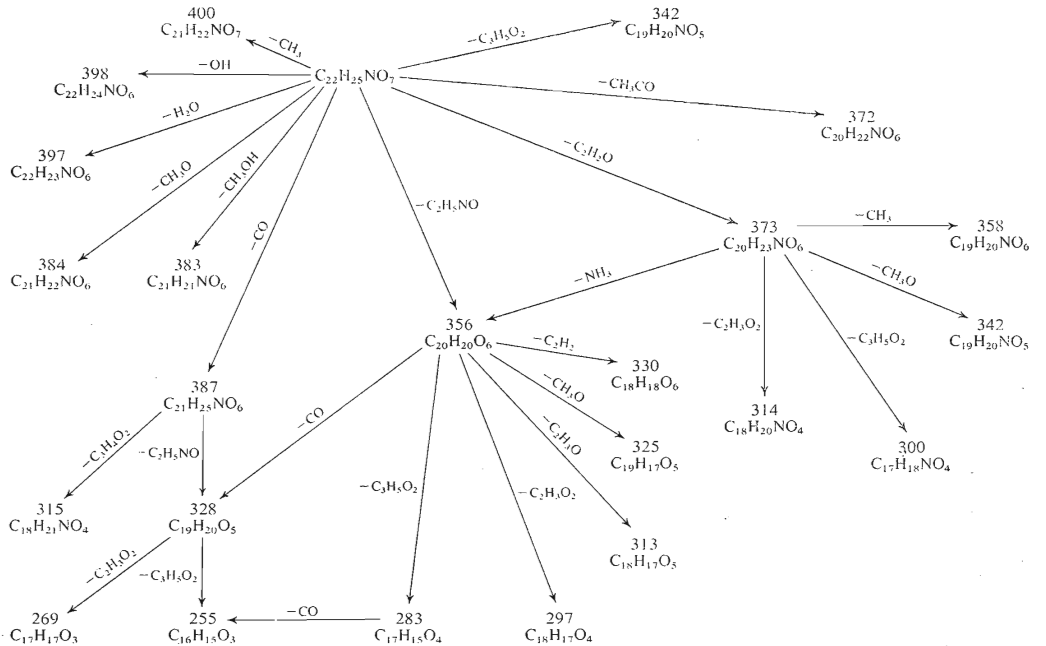


SCHEME 1. Mass Fragmentation Pattern of Epoxycolchicine *I*

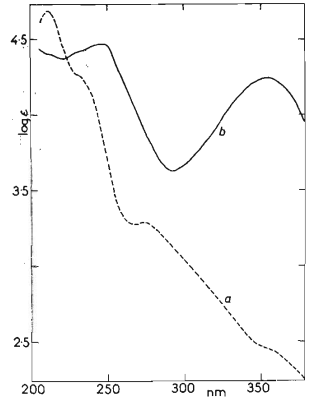


FIG. 1

Ultraviolet Spectra (in ethanol) of 10,11--Epoxycolchicine (*I*) (*a*) and Colchiciline (*III*) (*b*)

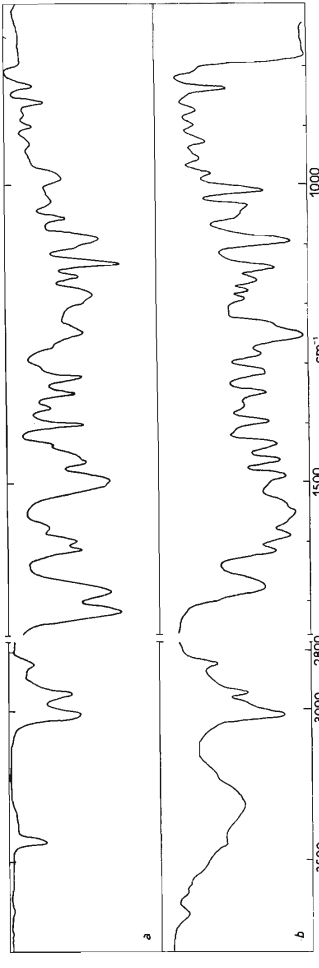


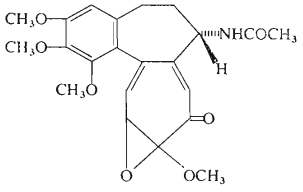
FIG. 2

Infrared Spectra (in chloroform) of 10,11--Epoxycolchicine (*a*) and Colchiciline (*b*)

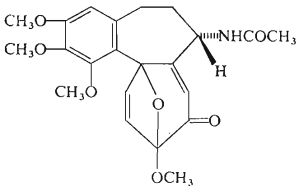
of four methoxyl groups (3·70, 3·80, 3·84, and 3·86 ppm), a singlet of one acetyl group at 1·85 ppm, the CH—NH grouping (multiplet at 3·06 ppm, a doublet at 5·47 ppm, $J_{CH,NH} = 6$ Hz), and two singlets of isolated protons at the sp^2-hybridized carbons at 6·20 and 6·44 ppm. Contrary to colchicine whose ^{1}H-NMR spectrum contains an AB system ($J_{AB} = 10{\cdot}6$ Hz, δ 6·90 and 7·38) of two tropolone protons, the spectrum of epoxycolchicine shows the presence of an AB system of different nature ($J_{AB} = 3$ Hz, δ 5·12 and 6·42). The stability of the UV spectrum (Fig. 1*a*) in alkaline medium and the IR spectrum (Fig. 2*a*) preclude the presence of the phenolic hydroxyl. The IR, UV (ref.[13]) and MS (ref.[11,12]) spectra show the presence of a carbonyl group which, however, is not part of the tropolone ring. The UV spectrum also indicates that the conjugation of the benzene ring differs from that of the tropolone colchicine alkaloids[8,13]. On the basis of the ^{1}H-NMR spectra, it has been concluded that the rings A and B are unchanged, whereas the ring C is of different nature.

Thus, a key point for the elucidation of the structure is the determination of the nature of the ring C. This ring bears one isolated olefinic proton, a keto group, a methoxyl group, two methine atoms constituting the above mentioned AB system and a so far unassigned oxygen atom bound to an oxygen bridge. The small coupling constant between the protons of the AB system precludes a vicinal coupling between the two olefinic protons. The large chemical shift difference also indicates that these protons belong to different types. We assume that these are one olefinic proton and one proton of the OCH type. The nuclear Overhauser effect observed between the signal of the methoxyl at 3·70 ppm and the signal of the OCH proton indicates their spatial proximity (*i.e.* most probably a vicinal arrangement since the chemical shift values of these two groups exclude the geminal alternative). Elimination of $C_2H_3O_2$ from some ions in the mass spectrum (Scheme 1) shows that one oxygen atom is bound to the same carbon atom as the methoxyl group. The location of the OCH proton near this methoxyl group (to explain the observed nuclear Overhauser effect) and a simultaneously bound oxygen atom to the carbon atom carrying this group should necessarily give rise to an epoxide ring. An epoxide ring must be present to explain simultaneously the proximity of the methoxyl group to the OCH type proton and the attachment of the oxygen atom to the carbon carrying this methoxyl. In view of the co-occurrence with colchicine, we assign the structure *I* (10,11-epoxycolchicine), consistent with all the above mentioned facts, to this new alkaloid. An examination of the Dreiding models shows that the distance of the methoxyl and the OCH proton is sufficient for the explanation of the nuclear Overhauser effect and that the ring C can exist in a conformation where the dihedral angle $H_{(10)}—C_{(10)}—C_{(11)}—H_{(11)}$ allows for $J_{vic} = 3$ Hz.

The mass spectrum of colchiciline (*III*) shows an intense molecular peak of *m/e* 415·1631 ($C_{22}H_{25}NO_7$). The high mass region of the spectrum contains the peak

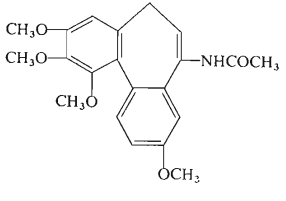


I

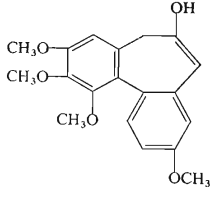


II

M − 18 and the small peaks M − 28 and M − 31. Expulsion of carbon monoxide is preferred in combination with elimination of water or acetamide; the resulting fragments *a*, $C_{21}H_{23}NO_5$ and *b*, $C_{19}H_{20}O_5$ (*m/e* 369 and 328, respectively) lose a methyl radical and yield the ions of *m/e* 354 and 313. Labelling of colchiciline with [O^2H]ethanol in the ion source proved two exchangeable hydrogens in the molecular ion (an alcoholic and an amidic one); the fragments M−18, *a*, and *b* are shifted of one mass unit only. The existence of competing fragmentations M → *a* and M → *b* indicates the presence of a hydroxyl group in the ring B of the colchicine skeleton.



a



b

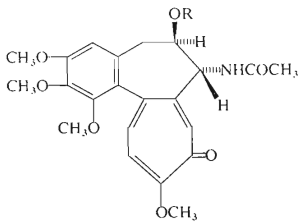
The ^{1}H-NMR spectrum ($CDCl_3$) of colchiciline corroborates the relationship to colchicine: it contains a singlet of one acetyl group (δ 1·96), four methoxyl groups (singlets 3·65, 3·90, 3·91, and 3·97 ppm), signals of four protons bonded to sp^2-hybridized carbon atoms consisting of an AB-system (δ 6·85 and 7·39, J_{AB} = 11 Hz), two singlets 6·66 and 7·44, and a broadened doublet of the NH proton (δ 8·11, J = 6 Hz). Double-resonance revealed that the splitting of the NH proton is caused by the proton δ 4·42 which by analogy with colchicine implies the presence of the CH—NH grouping. The nuclear Overhauser effect between the methoxyl δ 3·90

and a highfield member of the AB-system indicates again a spatial proximity of both groups, *i.e.* their vicinity on the same ring. In contrast to colchicine, the ^{1}H-NMR spectrum of colchiciline shows an additional one proton multiplet of the OCH type proton at 4·31 ppm and, in the higher upfield region, only one narrow two proton multiplet at 2·61 ppm instead of the signals of four protons. The spectrum measured in hexadeuteriodimethyl sulphoxide exhibits, in addition to all the above-mentioned characteristic signals, an exchangeable doublet at 5·69 ppm (J = 4·5 Hz) due to the secondary alcohol hydroxyl group proton. The noise decoupled ^{13}C-NMR spectra of colchicine and colchiciline contain separate signals of 22 carbon atoms. In the highfield region, the signal $C_{(6)}$ (30·0 t) is missing in colchiciline, instead of it there is a doublet (CH—O) at 60·2 ppm. This fact permits a definite localization of the secondary alcohol group at $C_{(6)}$. This conclusion is compatible with the other differences in the chemical shifts. On the basis of a thorough examination it leads to an assumption that the OH group forms a hydrogen bridge to the tropolone carbonyl and consequently that these two substances differ in the ring B conformation.

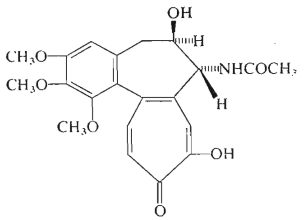
On an attempt to acetylate colchiciline with acetanhydride in the presence of anhydrous potassium acetate, the monoacetate *IV* has been obtained. In its mass spectrum there remain the ions *a* (*m*/*e* 369), *b* (*m*/*e* 328) and *a* − 15. A comparison of the mass spectrum of the ester *IV*, labelled in the ion source with [O^{2}H]ethanol, with that of colchiciline shows that the molecular peak shifts only by one atomic mass unit, so do the fragments *a* (M − CO—CH_3COOH) and *a* − 15; however, the position of the *b* (M − CO—CH_3CONH_2) ion remains unchanged. The IR spectrum of acetate exhibits ester frequencies at 1760 cm^{-1} (KBr), and 1732 ($CHCl_3$), respectively. The frequency of the hydroxyl group is missing. The ^{1}H-NMR spectrum of the ester *IV* exhibits singlets of two acetyl groups (δ 2·00 and 2·08), four methoxyl groups (3·68, 3·87, 3·94, and 3·98), and an AB-system (δ 6·84 and 7·39, J_{AB} = 10·5 Hz), two singlets of aromatic protons δ 6·46 and 7·52, and a doublet of the NH proton (δ 7·84, J = 6 Hz). The signal of the proton, vicinal to the NH proton (found by decoupling) yields a quartet at 4·66 ppm with splitting of 6 and 10 Hz. The larger coupling constant is caused by the proton δ 5·06 (splitting 10, 2·5, and 2·5 Hz). A downfield shift of this proton is consistent with the derivatization of a secondary alcohol group. The high value of the coupling constant indicates a quasi-antiperiplanar arrangement of the corresponding methine protons. Thus, it is possible to formulate the grouping —NH—CH—CH($OCOCH_3$)—CH_2—C= and colchiciline is attributable the structure of 6β-hydroxycolchicine (*III*). Thin-layer chromatography shows, however, that it is not identical with the previously described[14,15] alkaloid CC − 12 of the same structure.

Hydrolysis of colchiciline (*III*) with hydrochloric acid or with potassium hydroxide

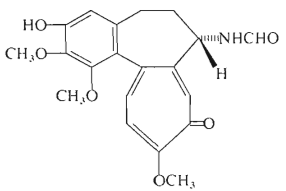
yields colchicileine (*V*) with identical IR and mass spectra. The latter exhibits a molecular ion at *m/e* 401 ($C_{21}H_{23}NO_7$) and the fragments M − H_2O (*m/e* 383) and M − CH_3CONH_2 (*m/e* 342), which evidences the presence of a hydroxyl and an acetamide group. The ^{1}H-NMR spectrum exhibits singlets of one acetyl group δ 2·00, three methoxyl groups (3·63, 3·90, and 3·92), two protons at 2·57 ppm, two one proton multiplets at 4·11 and 4·37 ppm, two singlets of aromatic protons at 6·65 and 7·62 ppm, an AB-system (δ 7·27, 7·63, J_{AB} = 12 Hz), and a broad singlet of the NH proton at 7·74 ppm. On the basis of a comparison with the ^{1}H-NMR spectra of colchicine, colchiceine, and isocolchicine[13], it is concluded that the $C_{(10)}$-methyl was lost and isomerization of the tropolone ring into the iso-series took place.



III, R = H
IV, R = $COCH_3$



V



VI

The structure of 3-demethyl-N-deacetyl-N-formylcolchicine (*VI*) was assigned on the basis of UV, IR, and ^{1}H-NMR spectroscopy[16] and mass spectrometry. 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[7].

EXPERIMENTAL

The UV spectra were measured in ethanol or in ethanolic sodium 0·05M hydroxide[17] on a UNICAM SP-700, the IR spectra in chloroform or in KBr disc on a Perkin-Elmer PE-567 or Zeiss UR-10, the ^{1}H-NMR spectra on the spectrometers Varian T-60 (60 MHz) and Tesla BS-487 (80 MHz), and the ^{13}C-NMR spectra on a spectrometer Jeol FX-60 (15 MHz). The mass spectra were registered in the instruments MS-902 and Varian MAT-311 (70 eV, direct inlet 190–220°C). The composition of all the ions was verified by high resolution measurements (peak matching, PFK standard). The metastable ions were followed up by the DADI (Direct Analysis of Daughter Ions[18]) method. The melting points were determined on a Kofler block and are not corrected.

Thin-layer chromatography was carried out on silica gel G in the systems S_1: benzene–ethyl acetate–diethylamine (5 : 4 : 1) + 4% methanol; S_2: chloroform–acetone–diethylamine (5 : 4 : 1) + 8% methanol. Detection in UV light and with Dragendorff reagent. hR_F Values and colour of the spots in UV light (in S_1 and S_2): Epoxycolchicine (*I*) 68, 81 (brown-beige); colchicine 56, 74 (beige); 2-demethylcolchicine 32, 52 (violet); 3-demethyl-N-deacetyl-N-formylcolchicine (*VI*) 25, 38 (violet); colchiciline (*III*) 23, 36 (yellow); 3-demethylcolchicine 21, 33 (violet).

10,11-Epoxycolchicine (*I*)

Obtained by column chromatography on Al_2O_3 of the mother liquors after lumicolchicine and colchicine[7]. It was eluted with the mixture ether–chloroform 1 : 1 and with chloroform. M.p. 251–253°C, $[\alpha]_D^{22}$ −237° ± 4° (*c* 1·68 in chloroform), reaction with conc. sulphuric acid brown-red. UV (ethanol) max 355 s, 273, 231 s, and 211 nm (log ε 2·45, 3·29, 4·27, and 4·69) (Fig. 1*a*). This spectrum does not change in ethanolic–alkaline medium. IR 1592, 1608, 1677 (N-acetyl), and 1712 cm^{-1} (nonconjugated carbonyl group) (Fig. 2*a*). The substance crystallized from ethyl acetate in prisms. Mass spectrum (*m/e* (relative intensity, composition)): M^+ 415 (26, $C_{22}H_{25}NO_7$), 387 (2·6, $C_{21}H_{25}NO_6$), 384 (1·9, $C_{21}H_{22}NO_6$), 374 (10), 373 (14, $C_{20}H_{23}NO_6$), 372 (6·4, $C_{20}H_{22}NO_6$), 358 (9), 357 (23), 356 (100, $C_{20}H_{20}O_6$), 342 (28, $C_{19}H_{20}NO_5$), 341 (15), 330 (13, $C_{18}H_{18}O_6$), 328 (16, $C_{19}H_{20}O_5$), 326 (11), 325 (17, $C_{19}H_{17}O_5$), 315 (24, $C_{18}H_{21}NO_4$), 314 (18, $C_{18}H_{20}NO_4$), 313 (19, $C_{18}H_{17}O_5$), 300 (16, $C_{17}H_{18}NO_4$), 297 (36, $C_{18}H_{17}O_4$), 283 (28, $C_{17}H_{15}O_4$), 269 (16, $C_{17}H_{17}O_3$), 255 (18, $C_{16}H_{15}O_3$).

Oxycolchicine (*II*)

Mass spectrum: M^+ 415 (83, $C_{22}H_{25}NO_7$), 384 (8), 383 (16), 374 (22), 373 (100, $C_{20}H_{23}NO_6$), 358 (20), 356 (49, $C_{20}H_{20}O_6$), 344 (11), 342 (24), 341 (18), 340 (9), 326 (17), 325 (23), 324 (65), $C_{19}H_{16}O_5$), 314 (26), 313 (56, $C_{18}H_{19}NO_4$), 310 (23), 300 (9), 298 (25), 288 (23), 286 (44), 284 (17), 283 (19), 282 (36), 281 (19).

Colchiciline (6β-Hydroxycolchicine, *III*)

Obtained by column chromatography on Al_2O_3 of the neutral chloroform extract. It was eluted with a mixture of chloroform–methanol (2%) after N-deacetyl-N-formylcolchicine and before a mixture of 3-demethylcolchicine and 2,3-didemethylcolchicine[7]. M.p. 170–171°C (decomp.), $[\alpha]_D^{22}$ −121° ± 4° (*c* 1·93 in chloroform); thin-layer chromatography shows that it is a rather polar substance. Reaction with conc. sulphuric acid is yellow, similar to that of colchicine. Contrary to colchicine derivatives with a free phenolic group on the ring A, it produces a yellow spot on silica gel or on paper. Its UV spectrum (Fig. 1*b*) is identical with that of colchicine and

does not change in ethanolic-alkaline medium. IR spectrum (chloroform) exhibits bands at 1582, 1592, 1620 (tropolone ring) and 1680 cm^{-1} (acetamido group) (Fig. 2*b*). Colchiciline crystallized from ethyl acetate in yellow crystals after having been left to stand for a long period at −30°C. Mass spectrum: M^+ 415 (86·2, $C_{22}H_{25}NO_7$), 397 (17·2, $C_{22}H_{23}NO_6$), 387 (7·6, $C_{21}H_{25}NO_6$), 384 (11·4, $C_{21}H_{22}NO_6$), 372 (34·5), 369 (69·0, $C_{21}H_{23}NO_5$), 356 (34·5), 354 (37·9, $C_{20}H_{20}NO_5$), 338 (19·7), 328 (100, $C_{19}H_{20}O_5$), 317 (58·6), 314 (51·7), 313 (51·0, $C_{18}H_{17}O_5$), 297 (48·3), 285 (65·5), 284 (65·5), 269 (63·8), 268 (60), 43 (100). ^{13}C-NMR spectra: colchicine 179·7 s, 170·5 s, 164·3 s, 153·7 s, 153·2 s, 151·3 s, 141·7 s, 137·4 s, 135·9 d, 134·6 s, 130·6 d, 125·8 s, 113·3 d, 107·6 d, 77·7 d, 61·7 q, 56·7 q, 56·3 q, 53·0 q, 36·4 t, 30·0 t, 22·8 q; colchiciline 179·5 s, 171·3 s, 164·3 s, 153·7 s, 151·1 s, 150·9 s, 142·0 s, 137·6 s, 136·3 d, 131·8 s, 131·4 d, 125·5 s, 113·7 d, 109·4 d, 75·3 d, 61·9 q, 61·5 q, 60·2 d, 56·7 q, 56·3 q, 38·9 t, 22·9 q. In these two spectra, multiplicity was established by Single Frequency OH-Resonance Decoupling experiment. The number and multiplicity (*i.e.* the degree of protonation) of signals with $\delta > 100$ ppm are in both compounds identical.

O-Acetylcolchiciline (*IV*)

Colchiciline (28 mg) was heated with acetanhydride and anhydrous potassium acetate at 50°C for 4 days. M.p. 150—151°C (ethyl acetate), hR_F (S_1) 73. Mass spectrum: M^+ 457 (19, $C_{24}H_{27}NO_8$), 442 (1·5), 429 (1·5), 415 (3, $C_{22}H_{25}NO_7$), 397 (4·4), 372 (3·3), 371 (3·3), 370 (11), 369 (39, $C_{21}H_{23}NO_5$), 368 (1·5), 354 (19), 351 (4·4), 344 (6·7), 340 (5·2), 338 (7·4), 328 (16), 326 (11), 312 (14), 295 (10), 268 (12), 254 (7·8), 43 (100).

Hydrolysis of Colchiciline (*III*) with Hydrochloric Acid

Colchiciline (10 mg) was heated on a boiling water bath with 10 ml of 0·1M-HCl for 1 h. After cooling, the solution was taken up into chloroform and the formed colchicileine (*V*) crystallized from methanol. M.p. 253—256°C; in S_1 remained on the start. The UV spectrum of this substance colchicileine (*V*) was identical with that of colchiceine in neutral and in alkaline media[19].

Hydrolysis of Colchiciline (*III*) with Sodium Hydroxide

Colchiciline (10 mg) was heated on a boiling water bath with 10 ml of 0·2N-NaOH for 2 h. After acidification with hydrochloric acid, the substance was taken up into chloroform, m.p. 252—256°C (methanol). On the basis of the mixed melting point and of the IR spectra, it was identical with colchicileine (*V*) which was obtained by the above-mentioned acidic hydrolysis. Mass spectrum: M^+ 401 (10, $C_{21}H_{23}NO_7$), 383 (5, $C_{21}H_{21}NO_6$), 370 (1·3), 359 (1·9), 358 (2·8), 357 (1·1), 355 (1·9), 343 (6·6), 342 (29, $C_{19}H_{18}O_6$), 341 (2·8), 340 (3·8), 329 (5·6), 314 (10), 300 (5·9), 299 (10), 298 (7·5), 271 (7·5), 270 (7·5), 255 (6·3), 254 (6·6), 241 (3·5), 240 (3·4), 239 (3·4), 227 (3·4), 212 (3·8), 211 (3·8), 43 (100).

3-Demethyl-N-deacetyl-N-formylcolchicine (*VI*)

On column chromatography on Al_2O_3, this alkaloid is eluted between 2-demethylcolchicine and colchiciline[7]. M.p. 263—267°C (ethyl acetate), $[\alpha]_D^{22}$ −180° ± 5° (*c* 0·65 in chloroform). Mass spectrum: M^+ 371 (31, $C_{20}H_{21}NO_6$), 343 (26, $C_{19}H_{21}NO_5$), 311 (42, doublet 1 : 1, $C_{18}H_{15}O_5$ and $C_{18}H_{17}NO_4$), 298 (82, $C_{18}H_{18}O_4$), 283 (100, $C_{17}H_{15}O_4$), 267 (85, $C_{17}H_{15}O_3$).

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