N-DEACETYL-N-3-OXOBUTYRYLCOLCHICINE, AN ALKALOID FROM *Colchicum autumnale* L. SEEDS*

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The structure of N-deacetyl-N-3-oxobutyrylcolchicine (*V*), based on spectroscopic measurements, was proposed for a minor alkaloid isolated from seeds of *Colchicum autumnale* L.

The isolation of alkaloids from seeds of *Colchicum autumnale* L. has already been described by us earlier[1]. By a modification of the isolation procedure, it was possible to obtain another pure compound from the amorphous fraction remaining after crystallization of N-formylcolchicine (*I*) and 3-demethylcolchicine (*III*). This communication deals with its structure elucidation.

The new alkaloid possesses the molecular formula $C_{24}H_{27}NO_7$ according to high resolution mass spectroscopic measurement of the molecular ion m/z 441. The resemblance of the UV spectra[2] to those of colchicine (*II*) suggests the same or very similar chromophore system. The base peak in the mass spectrum (m/z 312, $C_{19}H_{20}O_4$) and the ions m/z 340, 297, 281 and 269 allow a reliable identification as an N-acylated colchicine alkaloid[3-5]. Diagnostic loss of $C_4H_7NO_2$ from the m/z 413 ion ($M^+ - CO$) producing the base peak indicates that the side chain is of an increment C_2H_2O larger than that of colchicine[6]. The presence of a tropolone ring in the molecule is further substantiated (besides the UV spectra) by bands in the region of 1 628 to 1 565 cm^{-1} of the infrared spectrum, by elimination of CO from the molecular ion, by occurrence of an AB-system (7·13 and 7·44 ppm) in the ^1H NMR spectrum with coupling constant $J_{AB} = 10{\cdot}7$ Hz characteristic for vicinal tropolone protons[7] and by a carbonyl carbon signal at 164 ppm in the ^{13}C NMR spectrum[8,9]. The similarity to colchicine is confirmed also by its ^1H NMR spectrum. It contains a three-proton singlet of an acetyl group at 2·10 ppm, four methoxyl singlets at 3·67, 3·90, 3·93 and 3·98 ppm, one-proton multiplet at 4·99 ppm, two one-proton singlets

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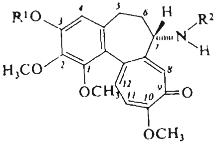
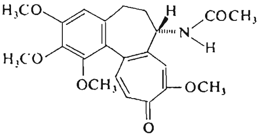
at 6·58 and 7·28 ppm, an AB-system (7·13 and 7·44 ppm, J = 10·7 Hz) and a doublet of an NH-proton at 7·31 ppm (J = 7·8 Hz). Homonuclear decoupling revealed a CH_2. .CHNH moiety. Compared with colchicine, the investigated compound exhibits one band more in its infrared spectrum (1 765 cm^{-1}, C=O), one two-proton singlet at 3·27 ppm in the ^1H NMR spectrum and signals of two carbon atoms at 56·1 (t)



I, $R^1 = CH_3$; $R^2 = CHO$
II, $R^1 = CH_3$; $R^2 = COCH_3$
III, $R^1 = H$; $R^2 = COCH_3$
IV, $R^1 = R^2 = COCH_3$
V, $R^1 = CH_3$; $R^2 = COCH_2COCH_3$

and 208·6 ppm (s) in the ^{13}C NMR spectrum. The difference in the molecular formula mentioned above was confirmed by elemental analysis. Therefore, the side chain of composition $C_4H_6NO_2$ contains in addition one carbonyl and one isolated methylene group. The amide group is retained (band at 1 655 cm^{-1} in the IR spectrum and a signal with δ_C 171·1 in the ^{13}C NMR spectrum). Thus the $C_{(7)}$ substituent is deduced as $NHCOCH_2COCH_3$. The two-proton singlet at 3·27 ppm can be assigned to its methylene group, the singlet at 2·10 ppm then represents the terminal methyl. Corresponding carbon atoms resonate at 171·1 ppm (amide carbonyl), 56·1 ppm (methylene), 208·6 ppm ($COCH_3$) and 22·6 ppm (CH_3CO). The difference in the side chain is reflected by chemical shift changes of the B and C rings carbons (Table I). Both possible tropolone ring arrangements – normal (II) and iso- (*VI*)



VI

are compatible with the observed facts. The latter have been found so far in synthetic compounds only (for a review see[10–13]). The first alternative is supported by the value of optical rotation[14], character of the UV spectrum[2], by a band at 1 592 cm^{-1} in the infrared spectrum (missing in the iso-series[2]) and by magnitude of the $J_{11,12}$ = 10·7 Hz coupling (which is usually larger[12] in the iso-series, 12·5–13 Hz). Final solution of this problem was achieved using the ^{13}C NMR spectrum. It contains in the case of colchicine (*II*) three signals around 150 ppm (C$_{(1)}$, C$_{(3)}$ and C$_{(7a)}$); but only two with isocolchicine (*VI*) (C$_{(1)}$ and C$_{(3)}$). Three signals observed in our case (Table I) allow to place our alkaloid to the normal series. The carbon chemical

TABLE I

Comparison of ^{13}C NMR spectra (δ-scale, 15·036 MHz, deuteriochloroform) of colchicine (*II*), (*II*), isocolchicine (*VI*) and N-deacetyl-N-3-oxobutyrylcolchicine (*V*)

Atom		*II*[a]	*V*	Δ$_1$[b]	*VI*[a]	Δ$_1$[c]
Ring A	1a	125·6	126·2	0·6	125·9	0·3
	1	151·1	151·3	0·2	150·8	0·5
	2	141·6	141·8	0·2	141·5	0·3
	3	153·5	153·3	−0·2	153·7	−0·4
	4	107·3	107·2	−0·1	107·6	−0·4
	4a	134·2	133·9	−0·3	134·9	−1·0
Ring B	5	29·9	29·9	0·0	30·1	−0·2
	6	36·2	33·8	−2·4	38·0	−4·2
	7	52·9	56·2	3·3	52·9	3·3
	7a	162·9	151·7	−1·2	145·6	6·1
Ring C	8	130·3	130·6	0·3	133·4	−2·8
	9	179·4	179·4	0·0	163·7	0·3[d]
	10	163·9	164·0	0·1	179·3	0·1[d]
	11	113·0	112·0	−1·0	111·2	0·8
	12	135·5	134·9	−0·6	141·7	−6·8
	12a	137·1	136·2	−0·9	134·9	1·3
OCH$_3$		61·5	61·6	0·1	61·3	0·3
		61·3	61·3	0·0	61·3	0·0
		56·1	56·1	0·0	56·1	0·0
		56·4	56·3	−0·1	56·1	0·2
Side chain		170·1	171·1	1·0	170·3	1·2
		22·6	22·6[e]	0·0	22·6	0·0
			56·1			
			208·6			

[a] Ref.[8]; [b] $\Delta_1 = \delta_V - \delta_{II}$; [c] $\Delta_2 = \delta_V - \delta_{VI}$; [d] difference calculated with interchange of C$_{(9)}$ and C$_{(10)}$; [e] terminal atom of the side chain.

shifts are in good agreement with that of colchicine in ring A, the differences are observed in the neighborhood of the site of change —$C_{(7)}$. The configuration at $C_{(7)}$ is *S*, judged from the sense of the optical rotation[15]. Therefore, the structure of the new alkaloid is *V*.

The most common acyl at nitrogen in colchicine alkaloids is acetyl; formyl (*e.g. I*, ref.[16]) and glycolyl[4,5] are also encountered. The 3-oxobutyryl represents a novel type of the side chain.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and were not corrected. UV spectra in ethanol or 0·05M ethanolic sodium hydroxide were measured on a UNICAM SP-700 spectrophotometer. Infrared spectra in KBr pellets were recorded on an Infragraf Mk. 3, H 1200 device. Mass spectra were obtained on a Varian MAT-311 instrument (ionizing energy 70 eV, ionizing current1 mA, ion source temperature 200°C, direct inlet at 150°C). Elemental composition of ions was determined by "peak-matching" technique (±5 ppm) using a perfluorokerosene standard. Metastable decompositions in the second field-free region were measured by scanning the electrostatic analyser voltage (Direct Analysis of Daughter Ions, DADI, ref.[6]). ^1H and ^{13}C NMR spectra were studied in deuteriochloroform at 25°C on a Jeol FX-60 spectrometer (59·797 and 15·036 MHz) working in the FT mode. Tetramethylsilane was used as an internal standard. Magnetic field of the spectrometer was stabilized by deuterium lock using the signal of the solvent. Chemical shifts were calculated from the digitally obtained address differences with accuracy ±0·005 and ±0·06 ppm (^1H and ^{13}C, respectively). Signal multiplicities were determined by off-resonance experiments.

Isolation

N-Deacetyl-N-formylcolchicine (*I*) and 3-demethylcolchicine (*III*) were obtained from the extract of *C. autumnale* seeds by crystallization.[1] Mother liquors were acetylated (acetanhydride, potassium acetate, 60°C, 4 days) and chromatographed (Al_2O_3 column, ether-chloroform 2 : 1). In the first place the compound *V*, and then 3-demethyl-3-acetylcolchicine (*IV*) (ether-chloroform 1 : 1) were eluted. The yield of *V* was about 500 mg per 20 kg of seeds. Under conditions described, neither colchicine (*II*) nor N-deacetyl-N-formylcolchicine (*I*) undergo acetylation.

N-Deacetyl-N-3-oxobutyrylcolchicine (*V*)

M.p. 222—223°C (ethyl acetate-ether), $[\alpha]_D^{25}$ $-194 \pm 4°$ (*c* 1·07, chloroform), hR_F 54 (colchicine hR_F 56) in the system benzene-ethyl acetate-dimethylamine-methanol 50 : 40 : 10 : 15. Yellow reaction with concentrated sulfuric acid. Gives a positive Overlin-Zeisel reaction only after heating with 3% hydrochloric acid. For $C_{24}H_{27}NO_7$(441·5) was calculated 65·37% C, 6·17% H, 3·18% N, 28·1% OCH_3; found: 65·21% C, 6·20% H, 3·19% N, 27·5% OCH_3. UV spectrum in ethanol λ_{max} (log ε): 237 sh (4·48), 243 (4·50), 352 nm (4·25); same spectrum upon addition of sodium hydroxide. Infrared spectrum: 1 618, 1 592, 1 565 (tropolone ring), 1 655 (CONH) and 1 765 cm^{-1} (C=O). Mass spectrum *m/z* (% of relative intensity, composition): 441 (12, $C_{24}H_{27}NO_7$, M^+), 413 (58, $C_{23}H_{27}NO_6$), 398 (13, $C_{22}H_{24}NO_6$), 385 (18, $C_{21}H_{23}NO_6$), 370 (43, $C_{21}H_{24}NO_5$), 356 (17, $C_{20}H_{22}NO_5$), 342 (17, $C_{19}H_{20}NO_5$), $C_{20}H_{24}NO_4$), 340 (19, $C_{20}H_{20}O_5$), 312 (100, $C_{19}H_{20}O_4$), 298 (32, $C_{18}H_{18}O_4$), 297 (31, $C_{18}H_{17}O_3$), 281 (26, $C_{18}H_{17}O_4$), 269 (15), 254 (15), 56 (77, C_3H_6N), 44 (69, C_2H_6N), 43 (65, C_2H_5N), 28 (100, CO).

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