2-DEMETHYLCOLCHIFOLINE - A NEW ALKALOID FROM COLCHICUM AUTUMNALE L.[1]

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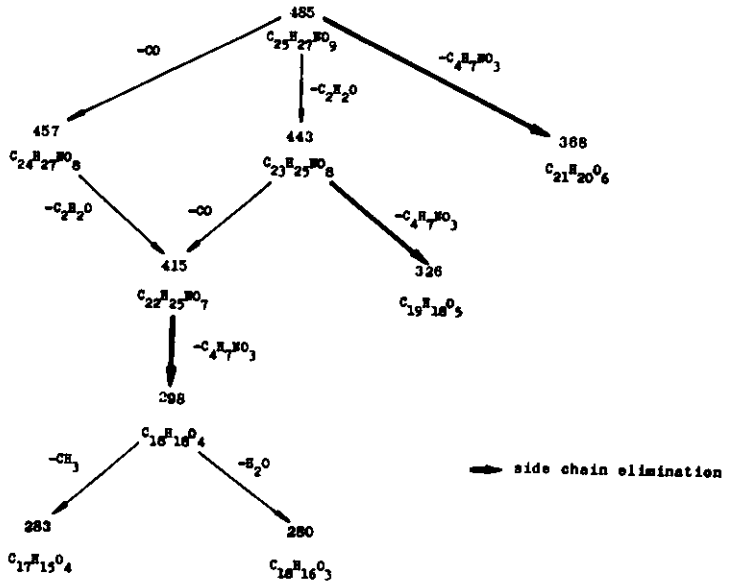
Dedicated to the Seventy-seventh Birthday of Professor Tetsuo Nozoe, Japan

Abstract - The structure of 2-demethylcolchifoline (IV), isolated from leaves of Colchicum autumnale L., has been determined on the basis of mass spectrometry, ^{1}H NMR and ^{13}C NMR spectroscopy.

From the leaves of Colchicum autumnale L., a highly polar fraction was obtained.[2] It could not be purified by column chromatography on Al_2O_3 or on silica gel and showed the presence of tropolone alkaloids. Gentle acetylation with acetic anhydride and anhydrous potassium acetate at 56° for 3 days followed by chromatography on Al_2O_3 gave a substance II of m.p. 229-232° (ethyl acetate), $[\alpha]_D^{23}$ -109° ± 3° (c = 0.68 in chloroform), hR_F 52 (silica gel F_{254} - solvent system: benzene - ethyl acetate - diethylamine - methanol (50:40:10:4)) (S_1). Color reaction with conc. sulfuric acid pink-yellow changing immediately into canary-yellow as in the case of colchicine. IR spectrum (KBr): peaks at 1763 (O-acetyl), 1686 (amide), 1619, 1600, 1588, and 1562 cm^{-1} (tropolone ring), UV spectrum (EtOH): 353 and 245 nm (log ε 4.29 and 4.53).[3]

On the basis of high resolution mass spectrometry, the empirical formula of this substance is $C_{25}H_{27}NO_9$. The molecular ion decomposes by loss of carbon monoxide, ketene and of the fragment $C_4H_7NO_3$. The latter group is also lost by the m/z 443 and m/z 415 ions (Scheme 1). Expulsion of the carbon monoxide is a typical feature of tropolones.[4] Elimination of the ketene is common in enol acetates whose genesis can be assumed on acetylation. Another characteristic feature of

fragmentation of N-acylated colchicine alkaloids is elimination of the corresponding acyl amide[5] which in our case corresponds to loss of the $C_4H_7NO_3$ group. The ^1H NMR spectrum contains two singlets of acetyl groups at σ 2.12 and 2.38, a four proton multiplet at σ 2.48, singlets of three methoxyl groups at σ 3.57, 3.87, and 3.99, a two proton singlet at 4.56, a one proton multiplet at σ 4.79, two singlets at σ 6.60 and 7.45, an AB system at σ 6.82 and 7.35 with J_{AB} = 11.2 Hz, and an exchangeable doublet (J=7.3 Hz) at σ 7.74. The integral of the spectrum corresponds to 27 protons. Double resonance revealed that the proton σ 4.79 is coupled both to the protons of the CH_2 group and the NH proton. Doublet structure of the NH proton means that on one side it is vicinal to an atom without protons. The spectrum of the new substance, contrary to that of colchicine (I), does not exhibit the singlet of a methoxyl at C-2 but it contains a signal of one acetyl group and a two proton singlet at σ 4.56. The ^{13}C NMR (Fig. 1 and Experimental part) exhibits signals of all 25 carbon atoms. Similarly to the ^{13}C NMR of colchicine it contains signals of carbons of two aliphatic methylene groups, three methoxyls, four sp^2-hybridized methines, four sp^2-atoms bound to carbon atoms, four sp^2-atoms bound to oxygen, one amide carbonyl (C-13) and one tropolone carbonyl (C-9). There are two methyl carbons in the ^{13}C NMR spectrum of II. However, their chemical shifts differ from that of C-14 in I. Significant differences were observed with three quaternary sp^2-hybridized carbons (two bonded to carbon and one to oxygen). The values of the proton carrying carbons remained nearly unchanged. Furthermore the spectrum of our acetylated substance contains signals of two ester carbonyls and of one oxymethylene (σ (C) 168.8, 166.9 and 62.9). Selective decoupling showed that the two protons in the singlet σ(H) 4.56 are bound to a carbon atom σ(C) 62.9. These data indicate that the arrangement of the three rings of the colchicine skeleton and the site of their substitution do not change. A difference is observed in the substitution of one methoxyl for an acetoxyl and in the modification of the side chain. Since, as in colchicine, the proton H-11 (σ 6.82) shows a long-range coupling to the methoxyl, the mentioned acetyl group must be located in ring A. No difference is also observed in the chemical shift of the C-1 methoxyl which due to shielding by the tropolone ring is shifted higher upfield. Between the positions 2 and 3 it can be decided with the help of ^{13}C NMR. Substitution of a methoxyl by an acetoxyl affects predominantly atoms in the



Scheme 1. Fragmentation of Compound II

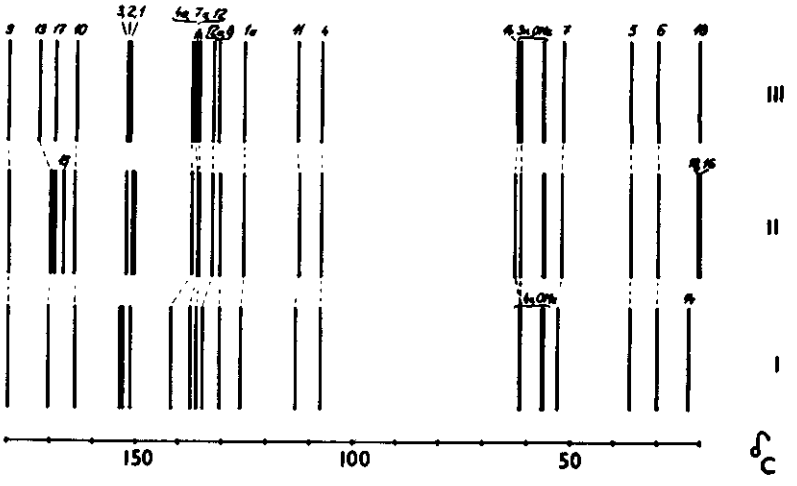
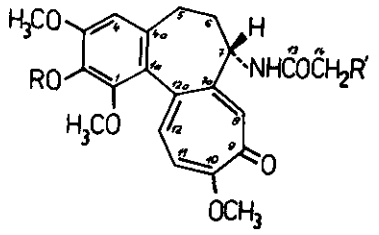


Fig. 1. Correlation of ^{13}C NMR Shifts of Colchicine (I), Diacetyl-2-demethyl-colchifoline (II), and 2-Acetyl-2-demethylcolchifoline (III)

ortho- and the para-position. The observed small change in the chemical shift of C-4 (δ 107.3) is only consistent when the acetoxyl is located at C-2. There has still to be clarified the structure of the side chain which can be ascribed the empirical formula $C_4H_6NO_3$. It must contain an NH group, two carbonyls, an isolated CH_2O group, and an acetylated methyl. To yield a doublet, the proton of the NH group must have a carbonyl in its vicinity. This accounts for the constancy of the chemical shift of the amide carbonyl in colchicine and in the substance II. There follows an oxymethylene group which is acetylated thus giving rise to the $NHCOCH_2OCOCH_3$ arrangement. This conclusion is confirmed by the behavior of the substance II in a slightly alkaline medium where the substance $C_{25}H_{25}NO_8$ (III) arises, hR_F 33 (S_1). Its ^1H NMR spectrum does not contain the signal δ(H) 2.12 and the two proton singlet is shifted to δ 4.08, which corresponds to deacetylation of the primary alcohol group. Similarly the ^{13}C NMR spectrum (Fig. 1), showing besides the absence of the signals of the carbon atoms of the acetyl group a well visible downfield shift of the signals of the amide carbonyl, is consistent with this conclusion. Consequently this new alkaloid is assigned the structure IV, its diacetyl derivative the structure II, and the product of gentle deacetylation the structure III. To our knowledge this is the first case of acylation of the amino group by glycolic acid in the colchicine series.



I	R = CH_3	R' = H
II	R = $COCH_3$	R' = $OCOCH_3$
III	R = $COCH_3$	R' = OH
IV	R = H	R' = OH

EXPERIMENTAL

Melting points have been determined on the Kofler block with an accuracy of $\pm 2^\circ$ and are uncorrected, the substances being dried for 1 hour at 100°/0.002 mm Hg. Optical rotation was measured on a polarimeter (Hilger), the UV spectra on a Unicam SP.700 in 95% ethanol, the IR spectra on a Perkin Elmer, model 567 in KBr pellets. Mass spectra were measured on a Varian MAT 311 spectrometer (70 eV, direct inlet 200°). High resolution measurements were carried out with the peak-matching technique (± 3 ppm) with perfluorokerosene as standard. Metastable

fragments were recorded with the help of changes in voltage in the electrostatic sector (DADI method). ^{1}H and ^{13}C NMR spectra were measured at 25° in $CDCl_3$ on a Jeol FX-60 instrument operating in the FT mode, internal standard TMS. Chemical shifts were calculated from digitally obtained address differences (accuracy 0.004 and 0.06 ppm) and are given in the δ scale.

Diacetyl-2-demethylcolchifoline (II). MS m/z (relative intensity in %, elemental composition): 485 (21.7, $C_{25}H_{27}NO_9$), 457 (5.1, $C_{24}H_{27}NO_8$), 443 (23.9, $C_{23}H_{25}NO_8$), 415 (37.6, $C_{22}H_{25}NO_7$), 397 (3.4, $C_{22}H_{23}NO_6$), 385 (2.9), 368 (12.0, $C_{21}H_{20}O_6$), 355 (12.0, $C_{20}H_{21}NO_5$), 340 (17.4, $C_{20}H_{20}O_5$), 326 (21.7, $C_{19}H_{18}O_5$), 324 (21.7, $C_{19}H_{16}O_5$), 318 (19.6), 312 (12), 308 (8.7), 299 (27.2), 298 (95.7, $C_{18}H_{18}O_4$), 297 (19), 283 (53.3, $C_{17}H_{15}O_4$), 280 (39.1, $C_{18}H_{16}O_3$), 267 (30.4), 44 (38.0), 43 (100), 32 (54.3).

^{13}C NMR (δ, off-resonance multiplicity): 20.4q, 20.7q, 30.0t, 36.3t, 52.1d, 56.1q, 56.4q, 61.5q, 62.9t, 107.3d, 112.4d, 125.1s, 130.6d, 132.4s, 135.4d, 135.7s, 137.0s, 150.4s, 150.9s, 152.3s, 164.3s, 166.9s, 168.8s, 169.8s, 179.5s.

2-Acetyl-2-demethylcolchifoline (III). This compound was prepared by dissolving diacetylcolchifoline (II) (20 mg) in methanol (4 ml) and addition of 0.1 N NaOH (1 ml). The solution was left standing for 24 hours at laboratory temperature, diluted to 10 ml with water and extracted with chloroform into which monoacetylcolchifoline (III) was taken up. The product did not crystallize from common solvents. $[\alpha]_D^{23}$ -112° ± 3° (c = 0.52 in chloroform).

^{1}H NMR (δ): 2.38s (3H), 2.48m (4H), 3.55s (3H), 3.87s (3H), 3.98s (3H), 4.08s (2H), 4.71m (1H), 6.61s (1H), 6.84 & 7.53 AB, J=11.2 Hz (2H), 7.58s, 7.75d (J=6.8 Hz, 1H).

^{13}C NMR (δ, off-resonance multiplicity): 20.5q, 30.1t, 36.4t, 51.8d, 56.3q, 56.4q, 61.5q, 62.3t, 107.4d, 112.8d, 125.2s, 131.0d, 132.3s, 135.5d, 136.2s, 137.1s, 151.5s, 151.7s, 152.2s, 164.1s, 168.9s, 172.6s, 179.7s.

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