Chemistry of Natural Compounds, Vol. 34, No. 6, 1998

AZIRIDINE AND HYDROXY- AND CHLOROETHYLAMINE DERIVATIVES OF COLCHICINE AND THEIR BIOLOGICAL ACTIVITY

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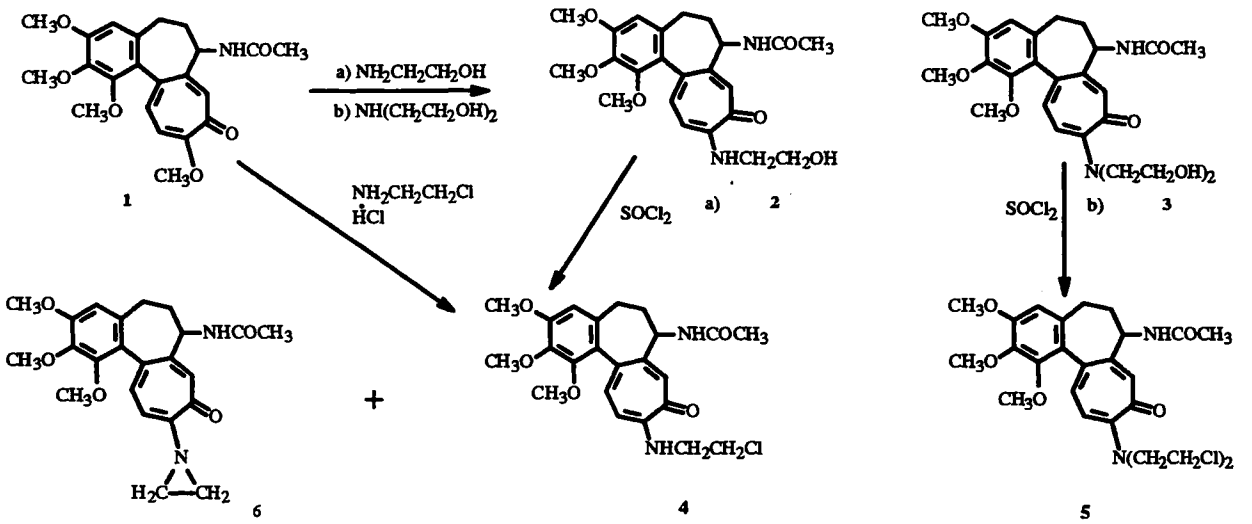
UDC 546.944.615

With the aim of enhancing the cytostatic properties of the initial alkaloid, new aziridine and bis(chloroethyl)amine derivatives of colchicine have been synthesized by the direct interaction of colchicine with chloroethylamine hydrochloride and also via the mono- and diethanolamine derivatives. The structures of the compounds obtained have been studied by spectral methods. An increased radiomodifying and antitumoral activity and a decreased toxicity of the substances synthesized as compared with the initial colchicine has been shown. Results obtained in the National Cancer Institute of the USA from the study of the cytostatic activity of the bis(chloroethyl)amino derivative of 60 tumor lines are presented.

A fall in toxicity in comparison with the initial alkaloids has been reported for a series of colchicine and colchamine derivatives [1, 2]; however, their antitumoral activity is not always retained. It is known that the introduction of alkylating fragments (aziridine, bis(chloroethyl)amine, etc.) into such carrier molecules as amino acids, nucleotides, sugars, hormones, and a number of others improves or ensures their antitumoral activity [3].

We have introduced aziridine and chloroethylamine substituents into the colchicine molecule with the aim of enhancing its antitumoral activity by combining the tubulin-interactive effect of colchicine and the alkylating action of the fragments introduced.

10-Chloroethylamino-10-demethoxycolchicine (**4**) was obtained by two methods: by the reaction of colchicine with chloroethylamine hydrochloride in an alkaline medium and from 10-hydroxyethylamino-10-demethoxycolchicine (**2**) by indirect chlorination.



Institute of Oncology and Radiology, Academy of Sciences of the Republic of Uzbekistan. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 782—789, November-December, 1998. Original article submitted May 1, 1998.

0009-3130/98/3406-0699$20.00

The interaction of colchicine with a fivefold excess of chloroethylamine hydrochloride and a fivefold excess of alkali (NaOH) formed four substances: colchiceine, (2), (3), and 10-aziridine-10-demethoxycolchicine (6). The formation of the aziridine derivative of colchicine (6) is connected with the conversion in an alkaline medium of chloroethylamine hydrochloride into aziridine [4], which then interacts with the colchicine. The hydroxyethylamino derivative of colchicine in this mixture is formed on the hydrolysis of one of the reaction products — the chloroethylamino (4) or the aziridine (6) derivative of colchicine, while colchiceine is formed in the hydrolysis of colchicine. After the chromatographic separation of the total products the yields of the individual substances obtained were, %: (4), 5.8; (2), 2.8; (6) 14.8; and colchiceine 37.4.

We selected the conditions of this reaction for a higher yield of substances (4) and (6) (see below), and with an increase in the excess of alkali to tenfold we obtained 30% of substance (4) and 50% of substance (6). The increase in the amount of alkali apparently led to the predominant formation of aziridine from the chloroethylamine, followed by its interaction with the initial colchicine. In this case the production of the aziridine derivative of colchicine competes with the alkaline hydrolysis of colchicine.

Thus, the yield of (4) in the direct interaction of colchicine with chloroethylamine hydrochloride does not exceed 30%, but this method enables substance (6) to be obtained without the preliminary conversion of chlorethylamine into aziridine. To confirm the structure of (6), it was obtained by the direct action on colchicine of aziridine synthesized from chloroethylamine hydrochloride by the action of alkali.

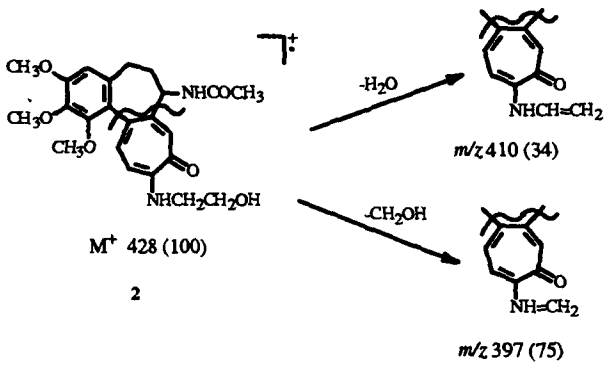
The yield when (4) was obtained through the intermediate amino alcohol derivative (2) was 60% (calculated on the initial colchicine). Since the second method is more rational, we obtained 10-β-bis(chloroethyl)amino-10-demethoxycolchicine (5) by the indirect chlorination of 10-β-bis(hydroxyethyl)amino-10-demethoxycolchicine (3).

The preparation of hydroxyethylamine derivatives of colchicine (substances (2) and (3)) is described in the literature [5, 6], and the conditions for performing the reaction and the melting points, yields, and elementary analyses of the products are given, but the absence of spectral characteristics and also the noncorrespondence of the results that we have obtained with those reported in the literature have impelled us to give ours. In particular, we obtained a melting point for compound (2) of 100—108°C (from ether), while [5] has 225—226°C (from ethyl acetate), and [6] has 189°C (from benzene); for (3), a lemon-yellow crystalline substance, we found mp 178—180°C, while in [5] (3) is described as an amorphous compound. Here we may recall the nonagreement of the constants given by a number of authors for a series of colchicine derivatives, using 10-amino-10-demethoxycolchicine as an example: 262—268°C [5]; 120—125°C (water) [7]; 128—131°C (methanol) [8]; 257—260°C (pentane) [9]; 120—122°C (methanol) [10]; 259—260°C [11]; 198—200°C (ethyl acetate) [12].

We obtained substance (2) with a yield of 82—90% by the action of a fivefold excess of monoethanolamine on colchicine, and substance (3) in a yield of 59% with an eightfold excess of diethanolamine.

A characteristic feature of the UV spectra of the compounds studied (2—6) is a bathochromic shift of the colchicine band at 230 nm by 25—35 nm and various shifts of the band at 350 nm (by 5—50 nm) with the appearance of a shoulder in the region of 380—420 nm and of new bands in the 200—220 nm region.

The IR spectra of the amino derivatives of colchicine lack the absorption band at 1250 cm^{-1} that apparently corresponds to the OCH_3 group in the tropolone ring, and a broad band appears in the 3200—3500 cm^{-1} region corresponding to the introduced amino and OH groups in the ethanolamine derivatives (2 and 3). In the spectra of (4) and (5), as compared with the hydroxy derivatives, chlorine bands have appeared at 780 and 670 cm^{-1} and the band at 3200—3600 cm^{-1} has greatly weakened.



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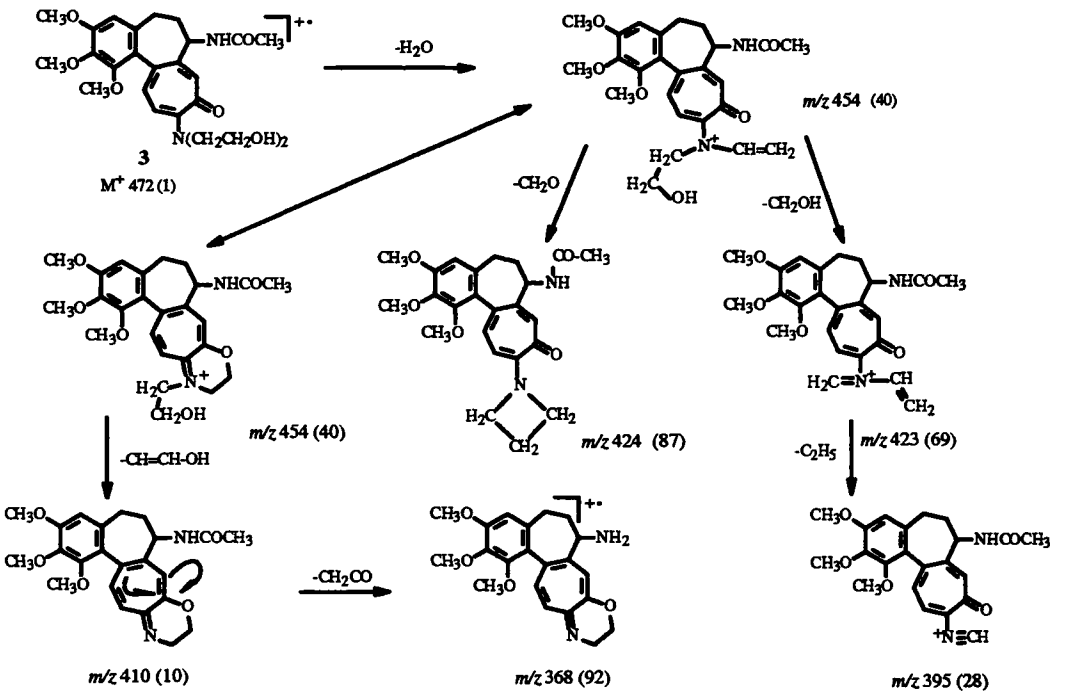
In the PMR spectra of the compounds synthesized, the signals of the OCH_3 group in the tropolone ring (4 ppm) has disappeared, while the general pattern of the signals of the aromatic protons characteristic of the initial colchicine and of the protons of the three methoxy groups of its benzene ring has been retained. The presence of hydroxymethyl groups is shown by a broad multiplet in the 3.5 ppm region, and methyleneamino groups are shown by a broad multiplet in the 2.3 ppm region.

The mass spectrum of (2) includes the maximum peak of the molecular ion (428), which loses water (*m/z* 410) or a hydroxymethyl fragment (*m/z* 397).



3

M^+ 472 (1)

In the mass spectrum of (3) we observed the peak of the molecular ion corresponding to the calculated molecular mass and peaks formed with the loss of a molecule of water and of a CH_2-OH fragment, having *m/z* 454 and 423, which shows the presence of two alcohol groups in the molecule. The further fragmentation of these ions is illustrated in the scheme. This type of fragmentation shows that in the molecules of amino derivatives of colchicine the positive charge is apparently localized predominantly in the region of the nitrogen atom of the tropolone ring.

The mass spectra of (4) and (6) are identical; for both substances the peak of a 411^+ ion appears, which for (6) corresponds to the molecular ion, and for (4) to a fragment from which an HCl molecule has split out. Their spectra are also close in the nature of their fragmentation.

The cytostatic activity of 10-β-bis(chloroethyl)amino-10-demethoxycolchicine (5) has been studied in the National Cancer Institute of the USA on panels of 60 lines of human tumors, on which the action of the compound was tested at five concentrations in tenfold dilution for 48 h. Subpanels (strains of definite varieties of tumors) were used for leukemia, non-small-cell lung cancer, cancers of the intestine and of the CNS, melanomas, and cancers of the ovaries, the kidneys, and the prostate and mammary glands. Figure 1 shows dose-response curves. Each curve is based on the percentage growth versus $\log_{10}$ of the corresponding concentrations for each cell line. The curves of the cell lines are grouped into subpanels. The horizontal lines correspond to the following values: at +50 — 50% suppression of the growth of the cell lines, GL_{50}: 0 — complete suppression of growth, TGL; -50 — 50% lysis of the cells, LC_{50}. The concentrations corresponding to the points of intersection of the curves with the horizontal lines are the GL_{50}, TGL, and LC_{50} values, respectively. Each concentration is expressed as $\log_{10}$ (molar or g/ml).

As can be seen from Fig. 1, the substance began to manifest activity in a concentration of 10^{-5} M (5.5 μg/ml), while in a concentration of 10^{-4} M (55 μg/ml) it completely inhibited the growth of tumor cells, with a subsequent cytocidic action. In an experiment with the transplantable animal tumors Walker's carcinosarcoma and sarcoma 45, substance (5) had a more pronounced action on the tumors than compound (4) and colchicine, suppressing their growth by 67 and 60%, respectively, while (4) did so by 60 and 24%, and colchicine by 46 and 33%.

TABLE 1. LD_{50} and Radiosensitizing Activity

Compound	LD_{50}, mg/kg	Survival rate at 800 r, %	Dose conversion factor
(1)	3.9	50	0.90
(2)	82	60	1.00
(2)hydrochloride	37	22	0.90
(3)hydrochloride	35	13	0.70
(4)	50	60	1.00
(5)	40	20	0.87
(6)	100	0	0.90

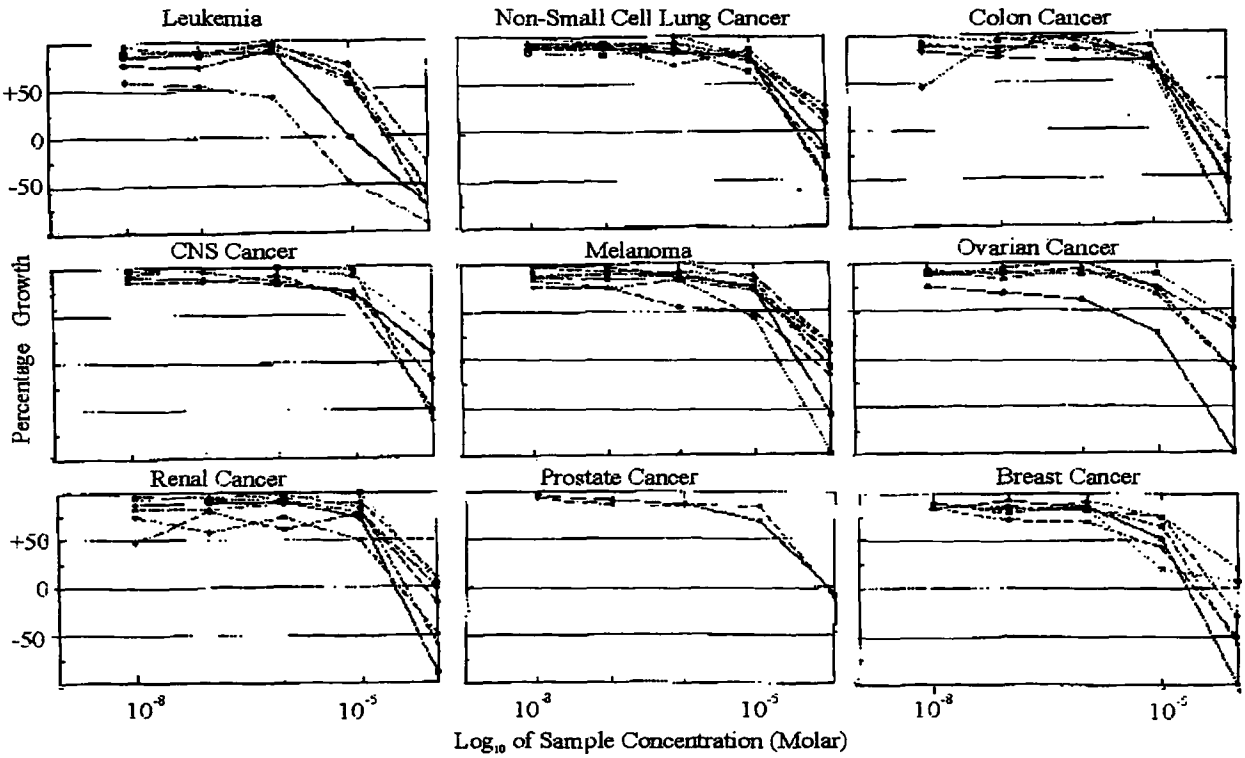


Fig. 1. Cytostatic activity of 10-bis(β-chloroethyl)amino-10-demethoxycolchicine on 60 human tumor lines.

Thus, substance (5) in a concentration of 10^{-4} M is capable of actively inhibiting growth and even of destroying the cells of many human tumors (in vitro); however, its toxicity in vivo (LD_{50} 40 mg/kg, MPD 3 mg/kg) does not permit the use of the optimum doses at which the maximum antitumoral activity is shown (55 mg/kg).

A study of the radiosensitizing activity of the compounds obtained (1—6) has shown that substances (2) and (5) are less toxic, but also less active, than their hydrochlorides.

Compounds (2) and (4) exhibited no radiosensitizing activity; the hydrochlorides of (2), (6), and (5), while having the same action as colchicine (1), showed a lower toxicity, and the hydrochloride of (3) proved to be a powerful radiosensitizer.

EXPERIMENTAL

UV spectra were taken on a Beckman SF-20 instrument in ethanol; IR spectra on a UR-10 instrument in paraffin oil and in KBr tablets; PMR spectra on a Varian XL-100 instrument in $CDCl_3$; and mass spectra on a MAT-311 instrument with direct injection of the specimen into the ion source. The mass spectra were interpreted with the help of P. B. Terent'ev (Moscow State University).

TLC was conducted in a fixed layer of alumina (ad. a), a fixed layer of silica gel LS 5/40 μm for TLC with 13% of gypsum (ad. b), and Silufol plates (ad. c). Solvent systems: 1) chloroform—methanol (24:1); 2) chloroform —benzene —acetone—methanol (20:5:4:3); 3) chloroform—benzene—acetone—methanol (20:5:4:8); and 4) chloroform —heptane—methanol (20:5.5:3.5). As spot reagents we used the Dragendorff reagent and iodine vapor.

10-Hydroxyethylamino-10-demethoxycolchicine (2). Monoethanolamine (0.73 ml) was added to colchicine (1 g) in 10 ml of alcohol, and the mixture was stirred at 70°C for 15 h. The resulting solution was then diluted with 100 ml of water and extracted with chloroform. The concentrated chloroform extract was transferred to a column of alumina, and the substances were eluted with chloroform and then with chloroform-alcohol mixture (1:1). After evaporation of the solvent, the amorphous mass was crystallized from dry ether. Substance (**2**) was isolated with a yield of 0.93 g (87%), mp 106—108°C (from ether), $[\alpha]_D^{20}$ -182° (*c* 0.5; ethanol), R_f 0.4 (system 2, ad. b), 0.64 (system 1, ad. a). Bright yellow microcrystalline substance readily soluble in alcohols, chloroform, and acetone; sparingly in water and ether.

Found: N 6.38%. Calculated: $C_{23}H_{38}N_2O_6$, N 6.54%.

UV spectrum (λ_{max}, nm): 205, 255, 370, 410.

IR spectrum (cm^{-1}): 1650 (CO of tropolone), 3270—3360 (NH + OH).

PMR spectrum (ppm): 1.96 (3H, s, N-Ac); 2.3+1.8 (4H, 2 br. s,-2H-5+2H-6); 3.52, 3.85, 3.88 (9H, 3 s, 3xOCH_3); 3.5 (2H, br. m, -N-CH_2); 3.8 (2H, br. m, CH_2-OH); 4.6 (1H, br. s, H-7); 6.46 (1H, s, H-4); 6.62 (1H, d, J=10.0 Hz, H-12); 7.30 (1H, s, H-11); 7.37 (1H, d, J=10.0 Hz, H-8); 7.90 (1H, t, NH).

Mass spectrum (*m/z*, %): 430 (14), 429 (61), M^+ 428 (100), 427 (6), 426 (6), 411 (28), 410 (34), 400 (19), 398 (28), 397 (75), 395 (25), 385 (28), 384 (11).

Hydrochloride of (2), mp 118—120°C (from acetone), $[\alpha]_D^{20}$ -220° (*c* 0.077; ethanol)[yield 78%]. Yellow crystalline substance, hygroscopic, readily soluble in water.

10-[Bis(β-hydroxyethyl)amino]-10-demethoxycolchicine (3). Diethanolamine (1.9 ml) was added to 1 g of colchicine in 10 ml of alcohol, and the mixture was stirred at 70°C for 20 h. Compound (**3**) was worked up in a similar way to (**2**) and was isolated with a yield of 0.93 g (59%), mp 178—180°C (from ether), $[\alpha]_D^{20}$ -70° (*c* 0.5; ethanol), R_f 0.25 (system 2, ad. b). Lemon-yellow crystals readily soluble in alcohols, chloroform, and acetone, and sparingly soluble in water and ether.

Found: N 5.91%. Calculated: $C_{25}H_{32}N_2O_7$, N 5.97%.

UV spectrum (λ_{max}, nm): 205, 255, 355.

IR spectrum (cm^{-1}): 1670 (CO of tropolone), 3270—3360 (NH + OH).

PMR spectrum (ppm): 2.0 (3H, s, N-Ac); 2.4 (4H, br.s,-2H-5+2H-6); 3.56, 3.84, 3.86 (9H, 3s, 3xOCH_3); 3.4 (2H, br. m, -N-CH_2); 3.8 (2H, br. m, CH_2-OH); 6.6 (1H, br. s, H-4); 6.9 (1H, d, J=11.0 Hz, H-12); 7.0 (1H, s, H-11); 7.2 (1H, d, J=11.0 Hz, H-8); 8.5 (1H, t, NH).

Mass spectrum (*m/z*, %): M^+ 472 (1), 455 (13), 454 (42), 424 (87), 423 (69), 411 (17), 410 (9), 395 (26), 369 (43), 368 (91), 365 (56), 338 (100).

Hydrochloride of (3) — red-brown hygroscopic crystals, mp 135—137°C, $[\alpha]_D^{20}$ -293° (*c* 0.077; ethanol), readily soluble in alcohols and in water.

10-(β-Chloroethylamino)-10-demethoxycolchicine (4). With stirring, 4 ml of thionyl chloride was added dropwise over 30 min to 1 g of 10-hydroxyethylamino-10-demethoxycolchicine hydrochloride (**2** HCl) in 5 ml of dry chloroform. Then the mixture was stirred without heating for 1 h and at 50—60°C for 7 h and was poured into water and extracted with chloroform. The (**4**) was purified on a column of alumina with elution first by chloroform and then successively with chloroform—alcohol mixtures in ratios of 10:1, 5:1, and 2:1. A substance with R_f 0.63 (Silufol, chloroform—methanol (24:1) system) was precipitated from concentrated chloroform solutions with dry ether. Yield 88% (0.92 g). Dark yellow crystals with mp 200°C (from ether), $[\alpha]_D^{20}$ -227° (*c* 0.5; chloroform), readily soluble in alcohols, chloroform, and acetone, sparingly soluble in water, ethyl acetate, and ether.

Found: N 5.60%. Calculated: $C_{23}H_{28}O_5N_2Cl_2$, N 6.54%.

UV spectrum (λ_{max}, nm): 220, 255, 380.

IR spectrum (cm^{-1}): weak 3210—3350 (NH); 1690 (CO-NH); 1630 (CO of tropolone); 1210, 1150, 1100, 1050, 1020 (vibrations of the tricyclic structure of colchicine); 780, 670 (Cl).

PMR spectrum (ppm): 1.96 (3H, s, N-Ac); 2.3+1.9 (4H, 2 br.s,-2H-5+2H-6); 3.56, 3.84, 3.86 (9H, 3 s, 3xOCH_3); 2.4 (2H, br.m, -N-CH_2); 3.8 (2H, br. m, CH_2-OH); 4.6 (1H, br. s, H-7); 6.45 (1H, s, H-4); 7.26 (1H, d, J=11 Hz, H-12); 7.49 (1H, s, H-11); 7.53 (1H, d, J=11 Hz, H-8).

Mass spectrum (*m/z*, %): 410 (100), 409 (3), 395 (10), 382 (6), 367 (14), 351 (13), 399 (9), 336 (10), 321 (6), 320 (6).

10-[Bis(β-chloroethyl)amino]-10-demethoxycolchicine (5). With stirring, 7 ml of thionyl chloride was added over 30 min to 1 g of 10-[bis(β-hydroxyethyl)amino]-10-demethoxycolchicine hydrochloride (**3** HCl) in 5 ml of dry chloroform. The reaction was performed in the same way as for the preparation of (**4**). After the excess of thionyl chloride had been distilled off, the mixture of products was separated on a column of alumina. The main reaction product (**5**), with R_f 0.18 (Silufol, chloroform—methanol (24:1) system) was isolated in an amount of 0.5 g (46%). Compound (**5**) is a yellow-brown powder having mp 140°C (with sublimation and decomposition), $[\alpha]_D^{20}$ -200° (*c* 0.46; ethanol), readily soluble in alcohols, chloroform, and Tween-80, moderately in benzene, and sparingly in water.

Found: N 5.38%. Calculated: $C_{25}H_{31}O_5N_2Cl_3$, N 5.15%.

UV spectrum (λ_{max}): 220, 265, 400.

IR spectrum (cm^{-1}): weak 3200—3300 (NH); 1680 (CO-NH); 1600 (CO of tropolone); 1180, 1150, 1100, 1050 (vibrations of the tricyclic structure of colchicine); 730 (Cl).

PMR spectrum (ppm): 2.00 (3H, s, N-Ac); 2.3+1.9 (4H, br.s,-2H-5+2H-6); 3.60, 3.84, 3.86 (9H, 3 s, 3xOCH_3); 2.4 (2H, br .m, -N-CH_2); 4-3.1 (4H, br. m, 2 CH_2-OH); 4.6 (1H, br. s, H-7); 6.35 (1H, s, H-4); 6.55 (1H, d, J=11 Hz, H-12); 7.3 (1H, s, H-11); 7.88 (1H, d, J-11 Hz, H-8).

10-Aziridine-10-demethoxycolchicine (6). A mixture of 1 g of β-chloroethylamine in alkaline solution (NaOH — 1 g) and a solution of 1 g of colchicine in alcohol was sealed into a tube. After two hours' boiling, the tube with the reactants was left in the dark for two days. The reaction mixture was then extracted with chloroform and the concentrated chloroform extract was purified on a column of alumina with elution by mixtures of chloroform and methanol in ratios of 24:1, 10:1, and 5:1, and by methanol. On fractionation we obtained 0.61 g (54%) of substance (**6**) with R_f 0.05 (system 2, ad. b), 0.33 (system 1, ad. a), and 0.34 g (30%) of substance (**4**) with R_f 0.18 (system 2, ad. b). Substance (**6**), mp 124 °C (from ether), $[\alpha]_D^{20}$ -391° (*c* 0.5; ethanol), yellow, with a microcrystalline structure. Readily soluble in alcohols, chloroform, acetone, and water, insoluble in ether.

Found: N 7.00%. Calculated: $C_{23}H_{26}N_2O_5$, N 6.83%.

UV spectrum (λ_{max}, nm): 210, 260, 360.

IR spectrum (cm^{-1}): 1680 (NHCO), 1610 (CO of tropolone).

PMR spectrum (ppm) : 1.99 (3H, s, N-Ac); 2.2 (4H, br.s,-2H-5+2H-6); 3.6, 3.84, 3.86 (9H, 3 s, 3xOCH_3); 3.16 (4H, br .m, -N-CH_2); 4.5 (1H, br. m, H-7); 6.66 (1H, s, H-4); 6.9 (1H, d, J=11.0 Hz, H-12); 7.56 (1H, s, H-11); 7.81 (1H, d, J-11 Hz, H-8).

Mass spectrum (*m/z*, %): M^+ 410 (100), 409 (3), 395 (10), 382 (6), 367 (14), 351 (13), 339 (9), 336 (10), 321 (6), 320 (6).

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