ACID-CATALYZED CYCLOCONDENSATION OF NITRILES. PART IV. SYNTHESIS AND SPASMOLYTIC ACTIVITY OF 1-SUBSTITUTED 3-AMINOISOQUINOLINES AND THEIR DERIVATIVES

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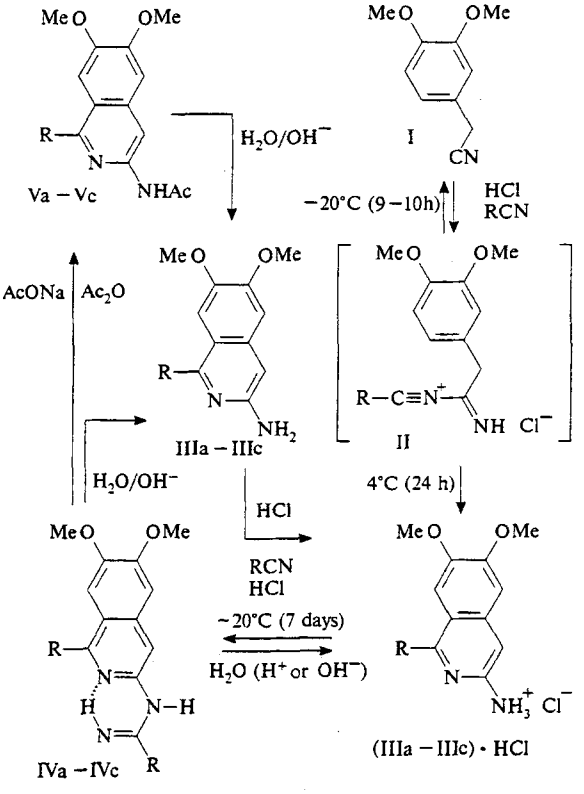
Biologically active compounds with an isoquinoline nucleus are widely used in medicine (papaverine, No-Spa, salsoline, etc.). Earlier, we have developed a simple and efficient method for the synthesis of 3-aminoisoquinolines and the corresponding 3-iminocarbonylamino derivatives [1, 2].

The purpose of this work was to obtain acyl derivatives of 3-aminoisoquinolines, substituted at the amino group, and study the spasmolytic activity of both 3-aminoisoquinolines and their derivatives.

The initial 3-aminoisoquinolines (IIIa – IIIc) were synthesized using a method described previously [1, 2]. The first step of this procedure consists in the acid-catalyzed cyclocondensation of arylacetonitriles. This is a three-stage process (see Scheme 1) involving the formation of intermediate N-imidoylnitrilium ions (II) and 3-aminoisoquinoline (III); the reaction mechanism was considered in detail elsewhere [1].

Despite a rather high yield of final 3-aminoisoquinolinyl aminoimides IV (50 – 72%), the products always contain unreacted initial nitriles and 3-aminoisoquinolines (dimeric products) [1, 2]. Below we will demonstrate that increasing the duration of the first stage leads to more complete consumption of the reagents. Chromatographic estimates show that the content of IIIa in the reaction mass eventually decreases to 3 – 5%, while the yield of amidine IVa, with an allowance for the conversion of 3,4-dimethoxyphenylacetonitrile, amounts to 87%. The content of 3-aminoisoquinolines in the reaction mixture varies depending on the conditions, reaching a maximum by the end of the low-temperature reaction stage [3]. However, taking into account difficulties encountered in the isolation of these compounds, it is expedient to terminate reaction upon the stage of 3-aminoimidoylisoquinolines IV, with their subsequent conversion into compounds III. Pure compounds III are most conveniently obtained through acetolysis of IV to N-acetyl derivatives (V), followed by their alkaline hydrolysis. The yields are almost quantitative in both stages. An alternative, theoretically possible, way of direct acidic hydrolysis of IV to III is not as convenient because compounds IV has proved to be highly

Scheme 1



R = 3,4-dimethoxybenzyl (IIIa, IVa, Va); benzyl (IIIb, IVb, Vb); methyl (IIIc, IVc, Vc).

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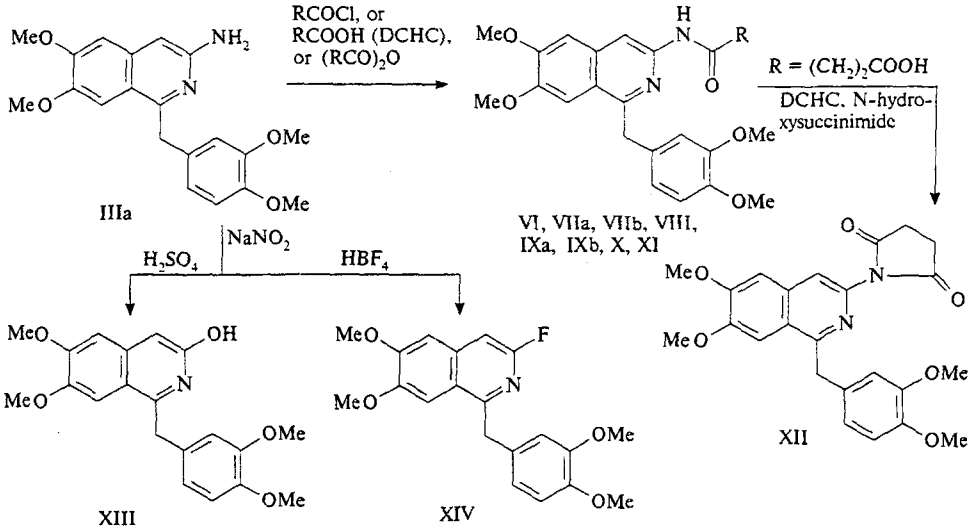
stable with respect to hydrolysis, which is rather unusual for amidines. The direct alkaline hydrolysis was justified only in the case of compound IVc (R = Me), probably because of its greater reactivity.

Cyclocondensation of 3,4-dimethoxyphenylacetonitrile I is performed in an inert solvent, usually dioxane. The cyclocondensation of I with liquid nitriles (phenylacetonitrile, benzonitrile, or acetonitrile) requires no solvent since the process is conducted with an excess of liquid nitrile.

Although the amino group at C-3 in isoquinolines possesses a lower pK_a (5.0) compared to isomeric aminoisoquinolines, the experimental data presented in this paper show that this amino group can well be used as a link in the directed synthesis of biologically active compounds. For example, this amino group in compound III enters into the acylation reaction under the action of acid chloroanhydrides and anhydrides and free acids in the presence of carbodiimides (Scheme 2). We used this reaction pathway to synthesize 3-nicotinoylaminopapaverine (VI) containing fragments of two spasmolytic molecules.

Scheme 2



R = 3-piridil (VI), $CH_2NHCOC_6H_4NO_2$-4 (VIIa), $CH_2NHCOC_6H_4NH_2$-4 (VIIb), $(CH_2)_2COOH$ (VIII), CH_2NH-BOC (IXa), $CH_2NH_2 \cdot CF_3COOH$ (IXb), $CH_2NHCO(CH_2)_3COOH$ (X), $(CH_2)_3COOH$ (XI)

We have also obtained a series of derivatives on the basis of compound IIIa, which are of interest as haptens used for the development of an original solid-state immunoenzymatic test kit for the determination of papaverine in biological liquids [4]. For this purpose, we have studied several variants of conjugation via an extended linking group. In the first case, 3-aminopapaverine and *para*-nitrohippuric acid under the action dicyclohexylcarbodiimide (DCHC) yielded 3-(4-nitrobenzoylglycilamino)isoquinoline (VIIa). Compound VIIa was reduced to amino derivative VIIb and then conjugated by dinitriding with bovine serum albumin (BSA). However, this way led to a conjugate with the degree of substitution corresponding to 5 hapten molecules per protein carrier molecule, which is insufficient for the analytical purposes. In the second variant, compound IIIa interacted with succinic anhydride to yield a succinyl derivative of 3-aminopapaverine (VIII). Compound VIII was converted into activated ester by the carbodiimide method to yield a cyclic product, 3-succinimidopapaverine XII, unsuitable for protein binding. According to a modified version, the reaction of compound IIIa with BOC-glycine in the presence of DCHC, followed by removal of the protective *tert*-butoxycarbonyl group, allowed us to obtain 3-N-glycilaminopapaverine (IXb). Compound IXb was successfully converted into the corresponding succinic acid monoamide (X) also capable of forming succinimide derivative upon conversion into activated ester. Glutaric acid has proved to be the most convenient spacer for the attachment of 3-aminopapaverine to protein. The glutaric acid monoamide derivative (XI) obtained from compound IIIa and glutaric anhydride is capable of forming, in the presence of DCHC and N-hydroxysuccinimide, the anticipated activated ester with a virtually quantitative yield. The activated ester formed a conjugate with BSA having a degree of substitution corresponding to 15 haptens per protein molecule [4].

Note that dinitriding of the compound IIIa in the presence of sulfuric acid leads to substitution of a hydroxy group for the amino group with the formation of 3-hydroxypapaverine (XIII), and the use of tetrafluoroboric acid yields 3-fluoropapaverine (XIV), which may also extend the possibilities of using aminoisoquinolines in the design of biologically active compounds.

EXPERIMENTAL CHEMICAL PART

The ^1H NMR spectra were measured on a Bruker WH-360 (360 MHz) and Varian HA-100D (100 MHz) spectrometers (USA) using TMS as the internal standard. The mass spectra were obtained on a Varian MAT CH8 instrument (Germany) with direct injection of samples into the ion source operated at an ionizing electron energy of 70 eV, and the temperature of the ionization chamber varied from 100 to 180°C. The IR spectra were recorded on a Specord-75 spectrophotometer. The melting points were determined on a Boethius heating stage (Germany). The purity of synthesized compounds was checked by TLC on the Silufol UV-254 plates eluted in a chloroform – methanol – 25% ammonia (950 : 50 : 1) system. 3-Aminopapaverine exhibited a bright blue fluorescence. The UV absorption by protein, hapten, and

antigen (conjugate) was measured on an SF-24 spectrophotometer (Russia) operated at a wavelength of 280 nm. The degree of substitution was calculated from the difference of the relative optical densities of the conjugate ($E_c^{0.1\%}$) and that of the initial protein ($E_p^{0.1\%} = 0.7$) with an allowance for the hapten absorption ($E_g^{0.1\%}$) as described in [4].

The main characteristics of the synthesized compounds are presented in Table 1. The data of elemental analyses coincided with the results of analytical calculations.

1-(3,4-Dimethoxybenzyl)-3-(3,4-dimethoxyphenylacetimidoylamino)-6,7-dimethoxyisoquinoline hydrochloride (IVa). A solution of 200 g (1.13 mole) of 3,4-dimethoxybenzylcyanide (I) in 250 ml of dry chloroform was cooled to – 20°C and saturated with 46 g (1.25 mole) of dry HCl for 9 – 10 h. Then the flask with the reaction mixture was kept for 24 h at 4°C and allowed to stand for 7 days at room temperature. The excess HCl and solvent were evaporated in vacuum. The orange powdered residue was extracted with hot benzene (300 ml) and diethyl ether (2 × 200 ml) in order to remove unreacted compound I and recrystallized from ethanol to obtain 165 g (78%) of hydrochloride IVa in the form of colorless crystals; m.p., 140 – 141°C (reported m.p., 139 – 140°C [3]; 140 – 142°C [2]). Mixing hydrochloride IVa with a 10% NaOH solution yielded a free base, which was separated by filtration and dried. The IR spectrum of the base in chloroform (ν_{max}, cm^{-1}): 3455 (NH), 3200, 2830 (OCH_3), 1605, 1585, 1555, 1495 (C=C, C=N), 1255 – 1200 (C–O–C), 1150, 1020 (C–O–C). The ^1H NMR spectrum of hydrochloride in DMSO-d_6 (δ, ppm): 3.68 (s, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 3.94 (s, 6H), 3.77 (s, 18H, 6 × OCH_3), 3.92 (s, 2H), 4.59 (s, 4H, 2 × CH_2), 6.88 (d, 1H, J 8.3 Hz), 6.91 (d, 1H, J 8.3 Hz, H-5′, H-5″), 6.78 (dd, 1H, J 2 and 8.3 Hz), 7.13 (dd, 1H, J 2 and 8.3 Hz, H-6′, H-6″), 6.98 (d, 1H, J 2 Hz), 7.14 (d, 1H, J 2 Hz, H-2′, H-2″), 7.36 (s, 1H, H-4), 7.58 (s, 1H, H-5), 7.66 (s, 1H, H-8), 10.17 (s, 1H), 10.62 (s, 1H), 12.72 (s, 3H, HCl, 2 × NH); mass spectrum (*m*/*z*, %): M^+ 531 (13), 514 (27), 354 (29).

General method for the synthesis of 1-substituted 3-iminocarbonylaminoisoquinolines using liquid nitriles. A solution of 200 g (1.13 mole) of 3,4-dimethoxybenzylcyanide (I) in the corresponding nitrile is cooled to – 20°C and saturated with 46 g (1.25 mole) of dry HCl for 9 – 10 h. Then the flask with the reaction mixture is kept for 24 h at 4°C and allowed to stand for 7 days at room temperature. The excess HCl and solvent were evaporated in vacuum. The powdered residue was extracted with hot benzene (300 ml) and diethyl ether (2 × 200 ml) in order to remove unreacted components and recrystallized from ethanol to obtain the corresponding amidine in the form of hydrochloride (see Table 1).

1-Benzyl-6,7-dimethoxy-3-phenylacetimidoylaminoisoquinoline hydrochloride (IVb). Molar ratio I / phenylacetonitrile = 1 : 15; yield 39.1%; IR spectrum of the base in chloroform (ν_{max}, cm^{-1}): 3470 (NH), 3200, 2820 (OCH_3), 1600, 1560, 1500 (C=C, C=N), 1465, 1425, 1400, 1220 (C – O – C), 1150, 870. ^1H NMR spectrum of the base in DMSO-d_6 (δ, ppm): 3.49 (s, 2H), 4.51 (s, 4H, 2 × CH_2), 3.86 (s, 3H), 3.87 (s, 6H, 2 × OCH_3), 7.10 – 7.41 (m, 15H, $H_{arom.}$, 2 × NH); mass spectrum (*m*/*z*, %): M^+ 411 (48), 394 (76), 294 (100).

3-(Acetimidoylamino)-1-methyl-6,7-dimethoxyisoquinoline hydrochloride (IVc). Molar ratio I / acetonitrile = 1 : 6; yield 64%; IR spectrum of the base in chloroform (ν_{max}, cm^{-1}): 3490 (NH), 3200, 2825 (OCH_3), 1610, 1585, 1560, 1490 (C=C, C=N), 1460, 1420, 1250 – 1200 (C–O–C), 950, 880. ^1H NMR spectrum of the hydrochloride in DMSO-d_6 (δ, ppm): 2.47 (s, 3H, C(=NH)CH_3), 2.90 (s, 3H, 1-CH_3), 3.95 (s, 3H), 3.96 (s, 6H, 2 × OCH_3), 7.34 (s, 1H), 7.38 (s, 1H), 7.49 (s, 3H, H-4, H-5, H-8), 10, 17 (s, 1H), 10.93 (s, 1H), 12.27 (bs, 3H, 2 × NH, HCl); mass spectrum (*m*/*z*, %): M^+ 259 (76), 242 (30), 218 (100).

3-Acetylamino-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline (3-acetylaminopapaverine) (Va). A mixture of 1.7 g (3.0 mmole) of hydrochloride IVa, 0.5 g anhydrous sodium acetate, and 10 ml acetic anhydride was boiled for 5 min, mixed with 30 ml benzene, and rapidly filtered through a heated filter. The residue was washed with 5 ml of hot benzene. The total filtrate was evaporated and the oily residue was dissolved on heating in 25 ml of ether and kept for 1 day at 4°C for crystallization. The crystals were filtered, washed with ether, and dried to obtain 1.12 g (98%) of compound Va in the form of colorless crystals; IR spectrum in chloroform (ν_{max}, cm^{-1}): 3415, 3300 – 3100 (several bands, NH), 2830 (OCH_3), 1680 (C=O), 1620, 1550, 1500 (C=C,

TABLE 1. Melting Temperatures and Empirical Formulas of the Compounds III – XIV

Compound	Empirical formula	M.p., °C
IIIa	$C_{20}H_{22}N_2O_4$	180 – 182 (ethanol)
IIIb	$C_{18}H_{18}N_2O_2 \cdot H_2O$	155 – 156 (ethanol)
IIIc	$C_{12}H_{14}N_2O_2 \cdot EtOH$	234 – 235 (ethanol)
IVa	$C_{30}H_{33}N_3O_6 \cdot HCl$	140 – 141 (ethanol)
IVb	$C_{26}H_{25}N_3O_2 \cdot HCl$	155 – 156
IVc	$C_{14}H_{17}N_3O_2 \cdot HCl \cdot H_2O$	270 – 274 (methanol)
Va	$C_{22}H_{24}N_2O_5$	160 – 161 (benzene)
Vb	$C_{20}H_{20}N_2O_3 \cdot H_2O$	180 – 181 (ethyl ether)
Vc	$C_{14}H_{16}N_2O_3$	211 – 212 (ethyl ether)
VI · H_2SO_4	$C_{26}H_{25}N_3O_5 \cdot H_2SO_4$	204 – 210
VIIa	$C_{29}H_{28}N_4O_8$	251 – 252 (dioxane)
VIIb	$C_{29}H_{30}N_4O_6$	260 – 262 (methanol)
VIII	$C_{24}H_{26}N_2O_7$	210 – 212 (dioxane)
IXa	$C_{27}H_{33}N_3O_7$	144 – 147
IXb	$C_{22}H_{25}N_3O_5$	–
X	$C_{26}H_{29}N_3O_8$	184 (with decomp.)
XI	$C_{25}H_{28}N_2O_7$	208 – 210 (ethanol – dioxane)
XII	$C_{24}H_{24}N_2O_6$	160 – 162 (dioxane)
XIII	$C_{20}H_{21}NO_5$	214 – 215 (ethanol)
XIV	$C_{20}H_{20}FNO_4$	109 – 110 (ethanol)

C=N), 1460, 1415,1280 –1200 (C–O–C), 1145, 1020 (C–O–C); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 2.24 (s, 3H, $COCH_3$), 3.37 (s, 3H), 3.82 (s, 3H), 3.87 (s, 3H), 3.94 (s, 12H, 4 × OCH_3), 4.41 (s, 2H, CH_2), 6.73 – 6.77 (m, 3H, $H_{arom.}$); 7.07 (s, 1H, H-5), 7.23 (s, 1H, H-8), 8.32 (s, 1H, H-4), 8.11 (s, 1H, NH); mass spectrum (*m*/*z*, %): M^+ 396 (100).

The other 3-acetylaminoisoquinolines were obtained by similar procedures.

3-Acetylamino-1-benzyl-6,7-dimethoxyisoquinoline (Vb). Yield of compound Vb, 89%; IR spectrum in chloroform (ν_{max}, cm^{-1}): 3420 (NH), 2830 (OCH_3), 1685 (C=O), 1620, 1600, 1580, 1500 (C=C, C=N), 1460, 1445, 1420, 1250 – 1200 (C–O–C), 1150, 1125; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 2.22 (s, 3H, $COCH_3$), 3.85 (s, 3H), 3.98 (s, 6H, 2 × OCH_3), 4.47 (s, 2H, CH_2), 7.07 (s, 1H, H-4), 8.31 (s, 4H, H-8), 7.18 – 7.30 (m, 6H, $H_{arom.}$ + H-5); 7.96 (s, 1H, NH); mass spectrum (*m*/*z*, %): M^+ 336 (100).

3-Acetylamino-1-methyl-6,7-dimethoxyisoquinoline (Vc). Yield of compound Vc, 64%; IR spectrum in chloroform (ν_{max}, cm^{-1}): 3420, 3300 – 3100 (several bands NH), 2830 (OCH_3), 1690 (C=O), 1625, 1555, 1500 (C=C, C=N), 1460, 1420, 1270 – 1200 (C–O–C); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 2.21 (s, 3H, $COCH_3$), 2.78 (s, 3H, CH_3), 4.0 (s, 6H, 2 × OCH_3), 7.06 (s, 1H), 7.16 (s, 1H), 8.26 (s, 3H, H-4, H-5, H-8), 8.23 (s, 1H, NH); mass spectrum (*m*/*z*, %): M^+ 260 (97), 245 (36), 218 (100).

3-Amino-1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline (3-aminopapaverine) (IIIa). A suspension of 1.2 g (3.03 mmole) of compound Va in 10 ml of ethanol was mixed with 8 ml of 25% KOH and boiled with reflux for 1 h. Then the reaction mixture was cooled and kept for 1 day at 4°C. The amorphous deposit was filtered to obtain IIIa at a quantitative yield; m.p., 180 – 182°C; IR spectrum of the base in chloroform (ν_{max}, cm^{-1}): 3490 (NH_2), 3400 (NH_2), 2835 (OCH_3), 1625, 1600, 1565, 1500 (C=C, C=N), 1415, 1240 (C–O–C), 1150, 1020 (C–O–C), 850; ^{1}H NMR spectrum of the base in $CDCl_3$ (δ, ppm): 3.78 (s, 3H), 3.81 (s, 3H), 3.83 (s, 3H), 3.95 (s, 12H, 4 × OCH_3), 4.31 (s, 2H, NH_2), 4.38 (s, 2H, CH_2), 6.56 (s, 1H, H-5), 7.16 (s, 1H, H-4), 6.73 – 6.83 (m, 4H, $H_{arom.}$ + H-8).

The other 3-aminoisoquinolines were obtained by similar procedures.

3-Amino-1-benzyl-6,7-dimethoxyisoquinoline hydrochloride (IIIb). Yield of compound IIIb, 96%; m.p., 129 – 132°C (ethanol – ether); IR spectrum of the base in chloroform (ν_{max}, cm^{-1}): 3480 (NH_2), 3395 (NH_2), 2830 (OCH_3), 1620, 1600, 1565, 1495 (C=C, C=N), 1470, 1415, 1250 – 1200 (C–O–C), 1150, 850; ^{1}H NMR spectrum of the base in $CDCl_3$ (δ, ppm): 3.80 (s, 3H), 3.95 (s, 6H, 2 × OCH_3), 4.37 (bs, 2H, NH_2), 4.46 (s, 2H, CH_2), 6.57 (s, 1H), 6.77 (s, 1H), 7.11 (s, 3H, H-4, H-5, H-8), 7.12 – 7.34 (m, 5H, $H_{arom.}$); mass spectrum of the base (*m*/*z*, %): M^+ 294 (100).

3-Amino-1-methyl-6,7-dimethoxyisoquinoline hydrochloride (IIIc). Yield of compound IIIc, 95%; m.p., 146 – 152°C (methanol – ether); IR spectrum of the base in chloroform (ν_{max}, cm^{-1}): 3490 (NH_2), 3395 (NH_2), 2830 (OCH_3), 1625, 1605, 1570, 1495 (C=C, C=N), 1470, 1420, 1250 – 1200 (C–O–C), 1150, 850; ^{1}H NMR spectrum of the base in $CDCl_3$ – CF_3COOH (δ, ppm): 2.87 (s, 3H, CH_3), 4.02 (s, 3H), 4,06 (s, 6H, 2 × OCH_3), 6.87 (s, 2H), 7.01 (s, 3H, H-4, H-5, H-8); mass spectrum of the base (*m*/*z*, %): M^+ 218 (100).

3-Nicotinoylaminopapaverine (VI). To a suspension of 20 g (0.0565 mole) of 3-aminopapaverine in 450 ml of dioxane mixed with 17.5 ml (14 g, 0.14 mole) of triethylamine was added dropwise a solution of 8 g (0.057 mole) of nicotinic chloroanhydride in 50 ml dioxane and the mixture was stirred at room temperature for 24 h. Then dioxane was evaporated and the residue was boiled with 150 ml of methanol and cooled to precipitate 24 g of hydrochloride. This precipitate was suspended in 70 ml of ethanol, boiled, and mixed (in the course of boiling) with 9 ml of concentrated sulfuric acid in 50 ml ethanol. The transparent solution was cooled and the lemon-yellow crystals that precipitated were filtered to obtain 23.4 g (90%) of compound VI.

1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-3-(4-nitrobenzoylglycilamino)isoquinoline (VIIa). To a solution of 0.354 g (1 mmole) of compound IIIa in 15 ml of absolute dioxane was sequentially added a solution of 0.3 g (1.45 mmole) of dicyclohexylcarbodiimide in 3 ml of dioxane and 0.25 g (1.1 mmole) of 4-nitrohippuric acid. The reaction mixture was allowed to stand for 12 h at room temperature and the residue of dicyclohexylurea (DCHU) was separated by filtration. The filtrate was evaporated to leave 0.52 g of a glassy residue used in the following stage without additional purification. If necessary, the product can be recrystallized from dioxane to obtain yellow crystals of compound VIIa; m.p., 251 – 252°C; IR spectrum in nujol mull (ν_{max}, cm^{-1}): 3400, 3280 (NH), 1690, 1655 (C=O), 1620, 1600, 1545 – 1510 (C=C, C=N, N–O), 1260 – 1220 (C–O–C); ^{1}H NMR spectrum of the base in DMSO-d_6 (δ, ppm): 3.67, 3.69, 3,83, 3.88 (s, 3H, 4 × OCH_3), 4.21 (d, 2H, J 7.2 Hz, NCH_2CO), 4.44 (s, 2H, CH_2), 6.77 (dd, 1H, J 2 and 8 Hz, H-6′), 6.82 (d, 1H, J 8 Hz, H-5′), 7.03 (d, 1H, J 2 Hz, H-2′), 7.22, 7.43, 8.22 (s, 1H, H-4, H-5, H-8), 8.14 (d, 2H, J 9.5 Hz, H-2″, H-6″), 8.35 (d, 2H, J 9.5 Hz, H-3″, H-5″), 9.17 (m, 1H, NH-1), 10.49 (m, 1H, NH-2).

3-(4-Aminobenzoylglycilamino)-1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline (VIIb). To a solution of 0.52 g (1 mmole) of compound VIIa in 30 ml of ethanol was added by portions 12 ml of concentrated HCl and 8 g of zinc dust. After 2 h, 20 ml of water was added and the reaction mixture was filtered. Then the solution pH was adjusted at 10 by adding 25% NH_4OH and the solution was extracted with chloroform. The extract was washed with water, dried over Na_2SO_4, and evaporated. The residue was crystallized from methanol to obtain 0.2 g (52%) of compound VIIb; IR spectrum in nujol mull (ν_{max}, cm^{-1}): 3485, 3445, 3360, 3255 (NH, NH_2), 1685 (C=O), 1640, 1630 (C=O), 1605, 1555, 1500 (C=C, C=N), 1255, 1230 (C–O–C); ^{1}H NMR spectrum

of the base in DMSO-d_6 (δ, ppm): 3.66, 3.67, 3,82, 3.88 (s, 3H, 4 × OCH_3), 4.07 (d, 2H, J 7.2 Hz, NCH_2CO), 4.41 (s, 2H, CH_2), 5.61 (s, 2H, NH_2), 6.57 (d, 2H, J 10 Hz, H-2″, H-6″), 7.61 (d, 2H, J 10 Hz, H-3″, H-5″), 6.76 (dd, 1H, J 2 and 8 Hz, H-6′), 6.80 (d, 1H, J 8 Hz, H-5′), 7.01 (d, 1H, J 2 Hz, H-2′), 7.20, 7.41, 8.20 (s, 1H, H-4, H-5, H-8), 8.36 (m, 1H, NH-1), 10.20 (s, 1H, NH-2).

Conjugate of 3-(4-aminobenzoylglycilamino)-1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline with BSA. To a solution of 10 mg of compound VIIb in 1 ml of dioxane and 0.5 ml of 0.4% HCl, cooled to 0°C, was added dropwise 0.3 ml of a 1.5% $NaNO_2$ solution. After 5 min, 0.1 ml of a saturated aqueous urea solution was added, the mixture was allowed to cool and mixed with a solution of 50 mg BSA in 3 ml of borate buffer (pH 9.18) and 0.5 ml dioxane. The reaction mixture was kept for 40 min at 0°C and then allowed to stand for 20 h at room temperature. The product was dialysed first against a dioxane – water (1 : 3) system, then against water, and lyophilically dried to obtain 0.031g of a brown powder of the VIIb – BSA conjugate with the degree of substitution corresponding to 5 hapten molecules per protein carrier molecule.

1-(3,4-Dimethoxybenzyl)-3-(N-*tert*-butoxycarbonylglycilamino)-6,7-dimethoxyisoquinoline (IXa). To a solution of 0.87 g (4.95 mmole) of BOC-glycine in 13 ml of dioxane was added a solution of 9.515 g (2.5 mmole) of DCHC in 7 ml of dioxane and the reaction mixture was stirred at 4°C for 1.5 h. Then DCHU was separated by filtration and the filtrate was mixed with a solution of 0.5 g (1.41 mmole) of compound IIIa in 70 ml of dioxane and allowed to stand for 24 h. After that the solution was evaporated and the residue treated with 25 ml of a 5% NaOH solution. The precipitate was filtered and dried to obtain chromatographically (TLC) pure compound IXa at a quantitative yield (0.75 g); ^{1}H NMR spectrum of the base in $CDCl_3$ (δ, ppm): 1.52 (s, 9H, Me_3COOC), 3.70, 3.75, 3,80, 4.00 (s, 3H, 4 × OCH_3), 4.42 (s, 2H, $ArCH_2$), 5.50 (t, 2H, $COCH_2NH$), 6.71 (m, 3H, $H_{arom.}$), 7.05, (s, 1H, H-5), 7.22 (s, 1H, H-8), 8.29 (s, 1H, H-4), 8.69 (bs, 1H, CH_2NHCO); mass spectrum (*m*/*z*, %): M^+ 511 (40), 437 (42), 354 (100).

3-Glycilamino-1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline trifluoroacetate (IXb). To 0.75 g (1.2 mmole) of amide IXa was added 2.0 ml of trifluoroacetic acid. After 2 h, dry ether was added and the precipitate was filtered and washed with ether to obtain trifluoroacetate IXb at a nearly quantitative yield (650 mg); ^{1}H NMR spectrum in CF_3COOH (δ, ppm): 4.01, 4.08, 4.26 (s, 12H, 4 × OCH_3), 4.90 (s, 2H, $ArCH_2$), 4.81 (m, 1H, $COCH_2NH^+$), 7.12 – 7.72 (m, 6H, $H_{arom.}$), 7.82 (bs, 1H, NHCO); mass spectrum (*m*/*z*, %): $[M + H]^+$ 412 (27), 395 (16), 354 (100).

1-(3,4-Dimethoxybenzyl)-3-(N-carboxypropionylglycilamino)-6,7-dimethoxyisoquinoline (X). To 0.9 g (1.21 mmole) of trifluoroacetate IXb in 30 ml dioxane was added 0.26 g (3 mmole) of sodium bicarbonate and the mixture was stirred for 12 h. The precipitate was separated by filtration. The filtrate was mixed with 0.18 g of succinic anhydride and boiled with reflux for 25 min. Then the solution was reduced in volume by evaporation to 10 ml, mixed with 10 ml of benzene, and cooled to 4°C. The precipitate was filtered, washed with benzene, and dried to obtain 0.67 g of compound X; mass spectrum (*m*/*z*, %): M^+ 511 (8), 493 (45), 395 (38), 381 (12), 354 (100).

1-(3,4-Dimethoxybenzyl)-3-carboxypropionylglycilamino-6,7-dimethoxyisoquinoline (VIII). To 0.88 g (2.48 mmole) of compound IIIa dissolved on heating in 30 ml of dioxane was added a solution of 0.26 g (2.6 mmole) of succinic anhydride in 3 ml of dioxane and the mixture was boiled with reflux for 30 min. Then the solution was reduced to half the initial volume by evaporation in vacuum, and mixed with 10 ml of benzene. The precipitate was filtered to obtain pale-yellow crystals of compound VIII at a 98% yield; m.p., 210 – 212°C; IR spectrum in chloroform (ν_{max}, cm^{-1}): 3470 (OH, NH), 2835 (OCH_3), 1710 (C=O), 1620, 1600, 1565, 1500 (C=C, C=N), 1460, 1415 (C–O–H), 1245 – 1200, 1175 (C–O–C).

1-(3,4-Dimethoxybenzyl)-3-carboxybutanoylamino-6,7-dimethoxyisoquinoline (XI). Compound XI was obtained in the form of colorless crystals by a similar procedure using compound IIIa and glutaric anhydride; yield, 63%; m.p., 208 – 210°C; mass spectrum (*m*/*z*, %): M^+ 468 (10), 467 (8), 450 (3), 382 (12), 354 (100); the procedure was described in detail elsewhere [2].

1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-3-succinimidoisoquinoline (XII). A mixture of 0.23 g (0.507 mmole) of compound VIII and 0.06 g (0.521 mmole) of N-hydroxysuccinimide was dissolved in 10 ml of boiling dioxane, rapidly cooled to 20°C. To this solution was added 0.12 g (0.583 mmole) DCHC and the mixture was allowed to stand overnight at room temperature. On the next day, 2 drops of glacial acetic acid (in order to decompose residual excess carbodiimide) and 5 ml of benzene were added to the reaction mixture. Then DCHU was separated by filtration and the filtrate was kept in a cool place until crystallization of compound XII in the form of colorless crystals; yield, 0.183 g (82%); m.p., 160 – 162°C; IR spectrum in nujol mull (ν_{max}, cm^{-1}): 1700 (C=O), 1635, 1610, 1565, 1500 (C=C, C=N), 1420, 1255, 1230, 1180, 1150, 1015 (C–O–C); ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 2.97 (s, 4H, $COCH_2CH_2CO$), 3.82, 3.84, 3,94, 4.00 (s, 3H, 4 × OCH_3), 4.54 (s, 2H, CH_2), 6.80 – 6.92 (m, 3H, $H_{arom.}$), 7.08, 7.38, 7.42 (s, 1H, H-4, H-5, H-8); mass spectrum (*m*/*z*, %): M^+ 436 (73), 421 (100).

1-(3,4-Dimethoxybenzyl)-6,7-dimethoxyisoquinolin-3-ol-(3-hydroxypapaverine) (XIII). To a mixture of 0.0436 g (0.123 mmole) of compound IIIa and 2 ml of 5% sulfuric acid, cooled to 0°C, was added dropwise (over 30 min) 3 ml of a 42% solution of sodium nitrite (0.183 mmole). The mixture was stirred for 30 min at room temperature and alkalized with a KOH solution. Then the mixture was filtered to remove contaminats and neutralized with acetic acid. The precipitate of compound XII was filtered, washed with water,

and recrystallized; m.p., 214 – 215°C; reported m.p., 216 – 218°C (water), 226 – 228°C (chloroform – ligroin) [1]; IR spectrum in nujol mull (ν_{max}, cm^{-1}): 1655 (C=O), 1595, 1555, 1520, 1490 (C=C, C=N), 1260, 1230, 1155, 1030 (C–O–C); the IR spectrum agrees with data reported in [6].

1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-3-fluoroisoquinoline (3-fluoropapaverine, XIV). To a solution of 0.05 g (0.141 mmole) of compound IIIa in 2 ml of THF and 2 ml of 40% tetrafluoroboric acid was added dropwise (over 10 min) a solution of 0.03 g (0.435 mmole) of sodium nitrite in 0.1 ml water at room temperature. The mixture was allowed to stand for 10 min at room temperature, alkalized with liquid ammonia solution, and extracted with chloroform. The chloroform extract was evaporated and purified on an Al_2O_3 column (containing 10 g of sorbent, having the degree of activity II, eluted with chloroform). The first collected fractions are evaporated to obtain 0.021 g (41.6%) of 3-fluoropapaverine (XIV) in the form of colorless crystals; IR spectrum in nujol mull (ν_{max}, cm^{-1}): 1620, 1600, 1585, 1560, 1510, 1500 (C=C, C=N), 1425, 1260, 1250, 1235, 1215, 1205 (C–O–C), 1160 (ArF), 1150, 1130 (C–O–C); ^{1}H NMR spectrum $CDCl_3$ (δ, ppm): 3.79, 3.82, 3.90, 4.01 (s, 3H, 4 × OCH_3), 4.52 (s, 2H, CH_2), 6.76 (d, 1H, J 8.7 Hz, H-5′), 6.83 (dd, 1H, J 1.3 and 8.7 Hz, H-6′), 6.87 (d, 1H, J 1.3 Hz, H-2′), 7.01, 7.03, 7.33, (s, 1H, H-4, H-5, H-8); mass spectrum (m/z, %): M^+ 357 (100), 342 (41), 178.5 (10).

EXPERIMENTAL BIOLOGICAL PART

The acute toxicity of the synthesized compounds was studied by the conventional method [7] using intraperitoneal injections into a group of mice weighing 18 – 20 g. The spasmolytic activity was studied by the Magnus method using samples of smooth muscles isolated from the thin intestine of rats [8]. The spasms were induced by acetylcholine (1×10^{-6} g/ml) and barium chloride (1×10^{-4} g/ml). The experiments were performed on an UGO Basilic setup (Italy) for the work with isolated organs. The substances were intro-

TABLE 2. Spasmolytic Activity and Acute Toxicity of 3-Aminoisoquinoline Derivatives

Compound	Concentration, g/ml	Acetylcholine spasm			Barium spasm			LD_{50}, mg/kg (i.p.)
		spasmolytic effect, %	p	MIC, %	spasmolytic effect, %	p	MIC, %	
IIIa · HCl	1×10^{-6}	62.5	< 0.001	$< 1 \times 10^{-6}$	41.2	< 0.001	1.2×10^{-6}	183 (160 ± 200)
	2×10^{-6}	60.2	< 0.001		68.3	< 0.001		
	1×10^{-5}	77.0	< 0.001		58.0	< 0.001		
IVb	1×10^{-6}	24.4	< 0.05	3.5×10^{-6}	46.2	< 0.001	6.3×10^{-7}	282 (240 ± 320)
	2×10^{-6}	46.4	< 0.002		40.0	< 0.001		
	1×10^{-5}	41.1	< 0.01		26.2	< 0.05		
IVa	1×10^{-6}	24.4	< 0.02	6.8×10^{-6}	2.6	> 0.5	1.3×10^{-5}	129 (100 ± 160)
	2×10^{-6}	30.0	< 0.02		5.5	> 0.5		
	1×10^{-5}	62.5	< 0.001		38.1	< 0.002		
IIIb · HCl	1×10^{-6}	9.4	> 0.25	1.5×10^{-6}	16.7	< 0.05	3.5×10^{-6}	129 (110 ± 150)
	2×10^{-6}	64.5	< 0.001		34.8	< 0.05		
	1×10^{-5}	91.2	< 0.001		85.5	< 0.001		
Va	1×10^{-6}	7.5	> 0.5	1.0×10^{-5}	1.2	> 0.5	2.4×10^{-5}	447 (370 ± 530)
	2×10^{-6}	11.5	< 0.05		4.9	> 0.5		
	1×10^{-5}	48.8	< 0.002		25.8	< 0.05		
IVb	1×10^{-6}	10.3	> 0.25	1.5×10^{-5}	0	> 0.5	$> 1 \times 10^{-5}$	70 (62 ± 80)
	2×10^{-6}	12.5	< 0.1		2.4	> 0.5		
	1×10^{-5}	35.8	< 0.001		16.7	0.05		
IIIc · HCl	1×10^{-6}	8.0	> 0.05	8.5×10^{-6}	2.2	> 0.5	$> 1 \times 10^{-5}$	14.1 (11 ± 17)
	2×10^{-6}	12.3	> 0.1		2.1	> 0.5		
	1×10^{-5}	56.0	< 0.001		6.0	> 0.25		
Vc	1×10^{-6}	4.8	> 0.25	1.3×10^{-5}	8.9	> 0.1	$> 1 \times 10^{-5}$	129 (100 ± 160)
	2×10^{-6}	12.5	> 0.05		8.6	> 0.1		
	1×10^{-5}	44.5	< 0.001		12.5	> 0.1		
Papaverine hydrochloride	1×10^{-6}	43.2	< 0.001	1.7×10^{-6}	33.4	< 0.002	3.0×10^{-6}	90 (80 ± 100)
	2×10^{-6}	55.9	< 0.001		40.5	< 0.01		
	1×10^{-5}	126	< 0.001		109.5	< 0.001		

duced to the test animals at the concentrations 1×10^{-6}, 2×10^{-6}, and 1×10^{-5} g/ml. Each concentration was tried in 6 – 13 tests. Papaverine hydrochloride was used as the reference drug. The experimental results were statistically processed by conventional methods.

As is seen from Table 2, the maximum activity on the model of acetylcholine-induced spasm was observed for 3-amino-1-benzyl-6,7-dimethoxyisoquinoline hydrochloride (IIIb) and 3-aminopapaverine hydrochloride (IIIa); much the same results were observed for the barium spasms. However, compounds IIIb and IIIa are less active as compared to papaverine hydrochloride; on the other hand, they are less toxic. Apparently, acylation of the amino group markedly reduces the toxicity, decreasing the spasmolytic activity as well. The maximum toxicity in the series studied was observed for the 1-methyl-3-amino derivative, which is probably explained by the absence of a benzyl fragment responsible for the spasmolytic effect.

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