as indicator until the color changed from red-violet to green. The result was calculated to barvinkan hydrochloride.

SUMMARY

A method has been developed for obtaining a stable 1% injection solution of barvinkan hydrochloride with the aid of a complex stabilizer.

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SOME REACTIONS OF α-HYDROXYMETHYLENE- AND α-DIMETHYLAMINOMETHYLENE-2,3-POLYMETHYLENE-3,4-DIHYDROQUINAZOLIN-4-ONES

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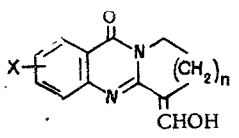
UDC 547.856.1

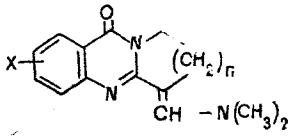
Many quinazoline derivatives have biological activities of various types. Thus, 2-perfluoro(chloro)alkyl-6,7-disubstituted 4-quinazolones possess a selective herbicidal action [1], and 2-alkyl-3-aryl-6(7)-substituted quinazolones are acaricides [2]. 2-Mono(di)alkylaminomethylene-1H(alkyl)-4-quinazolones and 2-tert-butyl-3-hydroxy-6-iodo-4-quinazolones have been proposed as fungicides [3, 4].

We have previously developed a method of synthesizing α-hydroxymethylene- and α-dimethylaminomethylene-2,3-polymethylene-3,4-dihydroquinazolin-4-ones and their 6- and 7-nitro derivatives (I, II), which are close analogs of the dialkylaminomethylene-4-quinazolones mentioned above, by the Vilsmeier–Haack formulation of 2,3-polymethylene-3,4-dihydroquinazolin-4-one [5].



I a-c

a) x=H, n=1
b) x=H, n=2
c) x=6—NO2, n=3



II a-c

a) x=H
b) x=6—NO2
c) x=7—NO2
n=1

In order to synthesize potential pesticides and to investigate the reactivity of the α-hydroxymethylene and α-dimethylaminomethylene groups we have studied some transformations of the above-mentioned compounds.

The α-hydroxymethylene- and α-dimethylaminomethylene-2,3-polymethylene-3,4-dihydroquinazolin-4-ones can be considered as enols and enamines. They should be capable of acylation

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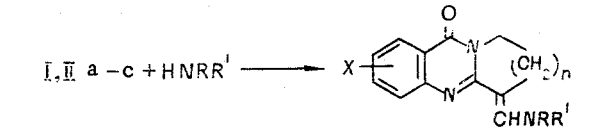
and also of reacting with primary and secondary amines. In fact, on the interaction of the α-hydroxymethylene-2,3-polymethylene-3,4-dihydroquinazolin-4-ones (Ia-c) with acetic, butyric, and benzoic anhydrides, acylation reactions took place readily with the formation of 2-acyl-(aroyl)oxymethylene-2,3-polymethylene-3,4-dihydroquinazolin-4-ones (IIIa-g). Compounds (IIIa, b, d, and e) were also obtained by heating the α-dimethylaminomethylene-2,3-polymethylene-3,4-dihydroquinazolin-4-ones (IIa-c) with the corresponding anhydrides at 100-110°C.

III a-g

a,d – g) R=CH_3; b) R=C_3H_7; c) R=C_6H_5; a-c,f) x=H; d,g) x=6–NO_2; e) x=7–NO_2; (a–e) n=1; f) n=2; g) n=3.

Compounds (Ia-c) also react with ammonia, primary amines (hydroxylamine, n-, iso-, and tert-butylamines, aniline, p-toluidine, phenylhydrazine, 2,4-dinitrophenylhydrazine) and secondary amines (dimethylamine, piperidine, morpholine) giving the enamines (IVa-p).

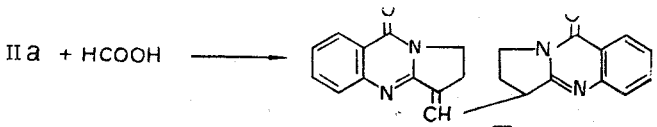


IV a-p

a) R=R′=H; b) R=H, R′=OH; c) R=H, R′=C_4H_9–n; d) R=H, R′=C_4H_9–iso; e) R=H, R′=C_4H_9– tert; f) R=H, R′=C_6H_5; g) R=H, R′=C_6H_4–CH_3–p; h) R=H, R′=NHC_6H_5; i) R=H, R′=$NHC_6H_3(NO_2)_2$–2,4; j) R=R′=CH_3; k) RR′=$(CH_2)_5$; l) RR′=$(CH_2)_2O(CH_2)_2$; m) RR′=$(CH_2)_5$; n) RR′=$(CH_2)_2O(CH_2)_2$; o) RR′=$(CH_2)_5$; p) RR′=$(CH_2)_2O(CH_2)_2$; a-l., p) x=H; m–n) x=6–NO_2; o) x=7–NO_2; a–o) n=1; p) n=2.

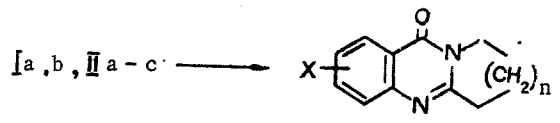
It is known that amines and enamines smoothly undergo the transamination reaction [6, 7]. It was found that compounds (IIa-c) react with the amines mentioned to give compounds (IVk, m-o).

To reduce the double bond of the methylene group of (IIa), we heated it in 85% formic acid and found that under these conditions compound (V) was formed in addition to the expected α-dimethylaminomethyl-2,3-trimethylene-3,4-dihydroquinazolin-4-one.



V

The formation of (V) is apparently explained by the initial splitting off of the dimethylaminomethylene group in the acid medium with the production of 2,3-trimethylene-3,4-dihydroquinazolin-4-one. The latter then reacts with the α-dimethylaminomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one to give the condensation product (V). In order to confirm the correctness of this hypothesis, we hydrolyzed compounds (I) and (IIa-c) in acid and alkaline media. It was found that in all cases the hydroxymethylene or dimethylaminomethylene residue was split out with the formation of the corresponding 2,3-polymethylene-3,4-dihydroquinazolin-4-ones. The same phenomenon was observed in the reaction of α-hydroxymethylene-2,3-tetramethylene-3,4-dihydroquinazolin-4-one (Ib) with 33% aqueous dimethylamine: We isolated 2,3-tetramethylene-3,4-dihydroquinazolin-4-one (VId).



VI a-d

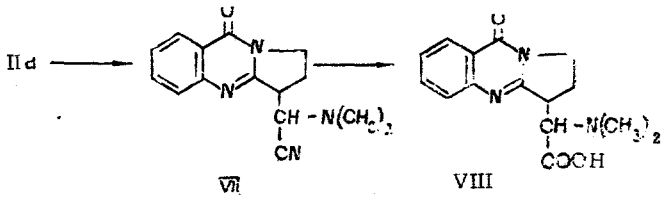
(a,d) x=H; b) x=6–NO_2; c) x=7–NO_2; (a–c) n=1; d) n=2.

TABLE 1. Characteristics of the Compounds Synthesized

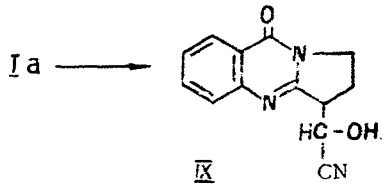
Initial compound	Reaction product	R	R'	Yield, %	mp, °C*	R_f†	Mass-spectr. mol. wt.	Empirical formula
Ia	IIIa	CH_3	—	58	214–216	0,63	256	$C_{14}H_{12}N_2O_3$
Ia	IIIb	$CH_3CH_2CH_3$	—	40	314–315	—	—	$C_{16}H_{16}N_2O_3$
Ia	IIIc	C_6H_5	—	87	213–215	—	—	$C_{19}H_{14}N_2O_3$
IIa	IIIa	CH_3	—	67	215–218	0,62	—	$C_{14}H_{12}N_2O_3$
IIa	IIIb	$CH_3CH_2CH_3$	—	15	314–316	—	—	$C_{16}H_{16}N_2O_3$
Ib	IIIf	CH_3	—	85	219–220	0,62	—	$C_{15}H_{14}N_2O_3$
IIb	IIId	CH_3	—	83	289–291	0,60	—	$C_{14}H_{11}N_3O_5$
Ia	IVa	H	H	81	126–128	0,28	—	$C_{12}H_{11}NO$
Ia	IVb	H	OH	50	187–190	—	—	$C_{12}H_{11}NO_2$
Ia	IVc	H	C_4H_9-n	39	179 181	0,65	—	$C_{16}H_{19}NO$
Ia	IVd	H	C_4H_9-iso	87	150–151	0,70	—	$C_{16}H_{19}NO$
Ia	IVe	H	C_4H_9-tert	44	145–147	0,80	—	$C_{16}H_{19}NO$
Ia	IVf	H	C_6H_5	85	205–207	—	289	$C_{18}H_{15}NO$
Ia	IVg	H	C_6H_4—CH_2-p	78	208–210	0 85	303	$C_{19}H_{17}NO$
Ia	IVh	H	NHC_6H_5	36	221–223	—	—	$C_{18}H_{16}N_4O$
Ia	IVi	H	$NHC_6H_3(NO_2)_2$—2,4	31	238–244	—	—	$C_{18}H_{14}N_6O_5$
Ia	IVj	CH_3	CH_3	97	177–179	0,61	241	$C_{14}H_{15}NO$
Ia	IVk	$(CH_2)_5$		65	180–181	—	—	$C_{17}H_{19}NO$
Ia	IVl	$(CH_2)_2O(CH_2)_2$		80	184–186	—	—	$C_{16}H_{17}NO_2$
IIa	IVk	$(CH_2)_5$		41	180–181	—	—	$C_{17}H_{19}NO$
IIb	IVm	$(CH_2)_5$		74	238–243	0,90	326	$C_{17}H_{18}N_4O_3$
IIb	IVn	$(CH_2)_2O(CH_2)_2$		87	257–259	0,80	—	$C_{16}H_{16}N_4O_4$
IIc	IVo	$(CH_2)_5$		74	247–249	0,92	326	$C_{17}H_{18}N_4O_3$
Ib	IVp	$(CH_2)_2O(CH_2)_2$		92	146–148	0 67	—	$C_{17}H_{19}NO_2$
IIa	V	—		58	245–248	0,68	382	$C_{23}H_{18}N_4O_2$
IIa	VII	—		54	138–140	0,57	268	$C_{15}H_{16}N_4O$
VII	VIII	—		78	206–208	0,54	—	$C_{15}H_{17}N_3O$
Ia	IX	—		67	306–308	—	241	$C_{13}H_{11}N_3O_2$
IIa	X	—		82	151–152	0,90	293	$C_{12}H_8N_2O_2Br$

*Compounds (IVa-h, j-p) and (X) were recrystallized from acetone; (IV, V, and VIb) from ethanol; (VII, VIII, and VIa and d) from hexane; and (IX) from acetone–hexane.
†For compounds (IIIa, VIa, c-e, g, j, m-p, V, and X) the values of R_f were determined in the chloroform–methanol (10:1) system (Silufol); and for (IIIf, d, IVp, VIa, b, d, VII, and VIII) in chloroform–ether (10:1) (alumina).

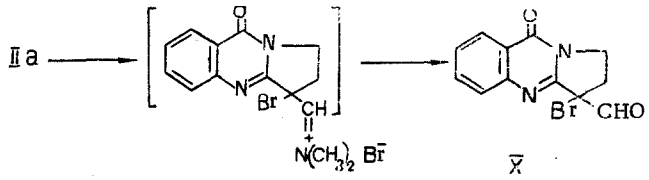
It appeared of interest to investigate some reactions involving addition to the double bond of the enamine group of α-dimethylaminomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one (IIa). Its reaction with acetone cyanohydrin gave cyano(dimethylamino)methyl-2,3-trimethylene-3,4-dihydroquinazolin-4-one (VII), which was hydrolyzed by concentrated hydrochloric acid to the amino acid (VIII).



The reaction of acetone cyanohydrin with (Ia) took place similarly, leading to the cyanohydrin (IX).



Enamines are intermediates formed in the production of α-bromo carbonyl compounds [8]. On studying the bromination of α-dimethylaminomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one, we obtained α-bromo-α-formyl-2,3-trimethylene-3,4-dihydroquinazolin-4-one (X), the formation of which obviously takes place via an intermediate immonium salt.



The structures of the compounds obtained were shown by the results of elementary analysis and IR, mass, and PMR spectroscopy, and their individuality by thin-layer chromatography (alumina or "Silufol").

In the IR spectra of (IIIa, b, d-g), the absorption bands of the ester carbonyl group appear in the 1760-1780-cm^{-1} region, and in the spectrum of (IIIc) at 1725 cm^{-1}. In the spectra of (Ia-c) the absorption band of the hydroxy group at 3300-3600 cm^{-1} has disappeared. The IR spectra of (IVa-i) are characterized by the presence of absorption bands in the 3300-3450-cm^{-1} region (ν_{NH}). In the spectra of compounds (VII) and (IX), the nitrile group appears at 2222 and 2204 cm^{-1}, respectively, and in (VIII) this band has disappeared and a new one has appeared at 1680 cm^{-1} (ν_{CO} of a carbonyl group).

The mass spectra of the compounds synthesized have the peaks of the molecular ions (18-100%) and also fragments corresponding to the proposed scheme for their fragmentation.

The yields and some physicochemical properties of the compounds obtained are given in Table 1.

A study of the pesticidal activity of the compounds under consideration, which was performed in the phytotoxicology laboratory of the Institute of Plant-Protecting Agents of the Academy of Sciences of the Uzbek SSR showed that the substances synthesized possess weak herbicidal activity and fungicidal activity.

EXPERIMENTAL

The results of the elementary analysis of all the compounds synthesized corresponded to the calculated figures.

α-Acetoxymethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one (IIIa). A solution of 0.1 g (0.4 mmole) of (Ia) or (IIa) in 3.24 g (32 mmole) of acetic anhydride was heated in a water bath for 1 h. Then it was cooled, and the brown crystals that deposited were filtered off, washed with acetic anhydride, and dried. This gave 0.08 g of (IIIa).

Compound (IIIa) was obtained similarly from (Ia) [sic].

α-Butyryloxymethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one (IIIb). A mixture of 0.1 g (0.4 mmole) of (IIa) and 1 g (6.3 mmole) of butyric anhydride was left at room temperature for 5-6 days. The white precipitate that had deposited was filtered off and washed with ether. This gave 0.02 g of (IIIb). Compound (IIIb) was obtained similarly from (Ia).

α-Benzoyloxymethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one (IIIc). A mxiture of 0.1 g (0.47 mmole) of (Ia) and 0.5 g (2.2 mmole) of benzoic anhydride was heated at 110-120°C for 1 h. The reaction mixture was cooled and treated with ether. The brown precipitate that deposited was filtered off and washed with ether. The yield of (IIIc) was 0.13 g; (IIId) was obtained from (IIb) and (IIIf) from (Ib) similarly.

α-Aminomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one (IVa). A mixture of 0.1 g (0.47 mmole) of (Ia) and 3-5 ml of 25% ammonia solution was heated in a water bath for 1 h. Then it was cooled, and the precipitate was filtered off, washed with water, and dried. Yield of (IVa) 0.08 g.

α-Hydroxyaminomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one (IVb). A solution of 0.1 g (0.47 mmole) of (Ia) in 2-3 ml of ethanol was treated with 0.1 g (3 mmole) of hydroxylamine.. After the mixture had been kept at room temperature for a day, it was extracted with chloroform. The organic layer was dried with Na_2SO_4 and the solvent was distilled off. Yield of (IVb) 0.07 g.

sec-Butylaminomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one (IVd). A solution of 0.1 g (0.47 mmole) of (Ia) in 0.5 ml of sec-butylamine was left at room temperature for 24 h. Then ether was added to the reaction mixture, and the precipitate was filtered off and was washed with ether. This yielded 0.11 g of (IVd). Compounds (IVc, e-i) were synthesized similarly.

Piperidinomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one (IVk). A mixture of 0.1 g (0.47 mmole) of (Ia) and 0.86 g (10 mmole) of piperidine was heated at 110-120°C for 1-2 h and was then left overnight, after which the excess of piperidine was evaporated off. The dry residue was treated with ether, and the precipitate that deposited was filtered off and washed with acetone. This gave 0.08 g of (IVk). Compounds (IV l-p) were obtained similarly.

Reaction of α-Dimethylaminomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-one with Formic Acid. A solution of 0.24 g (1 mmole) of (IIa) in 0.1 ml of 85% formic acid was heated in a water bath for 2.5 h. Then it was cooled and was treated with acetone, and the dark brown precipitate was filtered off, and washed with boiling acetone. This gave α-(2,3-trimethylene-3,4-dihydroquinazolin-4-one-α-ylmethylene)-2,3-trimethylene-3,4-dihydroquinazolin-4-one (V) with a yield of 0.11 g.

Hydrolysis of Compounds (I) and (II). a) A mixture of 0.1 g (0.47 mmole) of (Ia) and 4 ml of 5% caustic soda solution was heated in a water bath for 1 h and it was then cooled and was extracted with chloroform. The organic layer was dried with Na_2SO_4, and the solvent was distilled off. After recrystallization from hexane, 0.07 g (80%) of (VIa) with mp 110-111°C was isolated.

b) A mixture of 0.05 g (0.2 mmole) of (IIa) and 4 ml of 5% caustic soda solution was heated at 110-120°C for 2 h. Working up under the conditions described above gave 0.03 g (75%) of (VIa), identical with the 2,3-trimethylene-3,4-dihydroquinazolin-4-one obtained by method A.

c) A mixture of 0.1 g (0.44 mmole) of (Ia) with 0.2 ml of 33% aqueous dimethylamine was left for a day and was treated with ether. The precipitate that deposited was filtered off and recrystallized from hexane. This gave 0.04 g (45%) of (VId), mp 98-99°C. A mixture with 2,3-tetramethylene-3,4-dihydroquinazolin-4-one gave no depression of the melting point.

Acid Hydrolysis of (IIb). A solution of 0.3 g (1 mmole) of (IIb) in 10 ml of hydrochloric acid (1:1) was heated in a water bath for 4 h and was then left overnight and diluted with a fourfold amount of water. The precipitate that deposited was filtered off, washed with water, and dried. This gave 0.2 g (83%) of (VIb) with mp 186-188°C (methanol). A mixture with authentic 6-nitro-2,3-trimethylene-3,4-dihydroquinazolin-4-one gave no depression of the melting point.

α-[Cyano(dimethylamino)methyl]-2,3-trimethylene-3,4-dihydroquinazolin-4-one (VII). A mixture of 0.5 g (2 mmole) of (IIa) and 1.86 g (22 mmole) of acetone cyanohydrin was kept at room temperature for 14-18 h. Then it was treated with ether and the reaction product was purified by its passage through a column of alumina (eluent—chloroform). Yield of (VII) 0.3 g.

α-[Carboxy(dimethylamino)methyl]-2,3-trimethylene-3,4-dihydroquinazolin-4-one (VIII). Concentrated hydrochloric acid (0.24 ml) was added dropwise to 0.03 g (0.12 mmole) of (VII). The reaction mixture was left at room temperature for 1.5 h and was then heated on a water bath for 1 h, cooled, and diluted with water, and the precipitate that deposited was filtered off, washed with water, and dried. This gave 0.025 g of (VIII).

α-[Cyano(hydroxy)methyl]-2,3-trimethylene-3,4-dihydroquinazolin-4-one (IX). A mixture of 0.4 g (1.9 mmole) of (Ia) and 1.7 g (20 mmole) of acetone cyanohydrin was heated at 70-80°C for 30 min. The reaction mixture was cooled and dissolved in ether, and hexane was added. The precipitate that deposited was filtered off. Yield of (IX) 0.3 g.

α-Bromo-α-formyl-2,3-trimethylene-3,4-dihydroquinazolin-4-one (X). With cooling and vigorous stirring, a solution of 0.1 g (1.25 mmole) of bromine in 5 ml of chloroform was added dropwise to a solution of 0.1 g (0.4 mmole) of (IIa) in 3 ml of chloroform. The reaction mixture was stirred at room temperature for 1 h and was then left for 12 h. The residue after the solvent had been distilled off was treated with water. The crystals that deposited were separated off and washed with boiling acetone. Yield of (X) 0.1 g.

SUMMARY

By the reaction of α-hydroxymethylene- and α-dimethylaminomethylene-2,3-polymethylene-3,4-dihydroquinazolin-4-ones with acid anhydrides and primary and secondary amines, the corresponding α-acyloxymethylene- and α-mono(di)-substituted aminomethylene-2,3-polymethylene-3,4-dihydroquinazolin-4-ones have been synthesized. The addition of hydrocyanic acid (from

acetone cyanohydrin) to the double bond of the methylene group of the α-hydroxymethylene- and α-dimethylaminomethylene-2,3-trimethylene-3,4-dihydroquinazolin-4-ones has led to α-[cyano-(hydroxy)methyl]- and α-[cyano(dimethylamino)methyl]-2,3-trimethylene-3,4-dihydroquinazolin-4-ones.

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ISOLATION OF TRANSFERRIN FROM RAT BLOOD AND ITS PURIFICATION AND SOME PHYSICOCHEMICAL PROPERTIES

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UDC 576.8097.543.544

Transferrin (Tf — synonym: siderophilin) is a metal-containing glycoprotein which is responsible for the transport of iron into the cells of the erythroid series, where the biosynthesis of hemoglobin is performed. It consists of one polypeptide chain with a molecular weight of 70,000-90,000 daltons and has two specific sites binding two iron ions [2].

The transferrins form one of the most polymorphic systems of blood serum proteins. In man no less than 17 alleles forming an autosomic two-allele system controlling a single locus and inherited codominantly have been found. The molecular forms of transferrin differ in the magnitude of the charges, which depend on the number of sialic acid residues attached to the protein and, to a smaller extent, on the presence of one or two ferric iron ions attached to the protein molecule [1].

On electrophoresis in polyacrylamide gel (PAAG), the transferrins of different species of animals migrate in the form of several zones, which permits us to speak not of transferrin but of transferrins [4].

There is no information in the literature on the mechanism of the biosynthesis of this protein and its regulation at the molecular and genetic levels. We have isolated and partially characterized the transferrin from rat blood serum in order to study the molecular mechanisms of the regulation of its biosynthesis.

As a result of precipitation with ammonium sulfate and two subsequent stages of chromatography on ion-exchange resins (Fig. 1), the transferrin was purified to the state of electrophoretic homogeneity (Fig. 2b).

The molecular weight of the transferrin determined by electrophoresis in PAAG in the presence of sodium dodecyl sulfate (NaDDS) was 76,500 daltons, which agrees well with the molecular weight of this protein for many other species of animals and man [4-7]. The absorption spectrum in the visible region after rechromatography showed a peak at 465 nm, and there was a second peak with a maximum of 410 nm which appeared after chromatography on DEAE-Sephadex A-50, apparently representing a hemopexin impurity [6]. Isoelectric focusing in PAAG with the use of 40% ampholine (pH 3-10) showed two bands corresponding to two isoforms of transferrin (Fig. 2c), which indicates the polymorphic nature of this system of blood proteins

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