The values given in parentheses are calculated by least square method.

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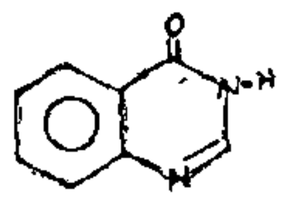
SYNTHESIS OF QUINAZOLINONE-4-N-MANNICH BASES

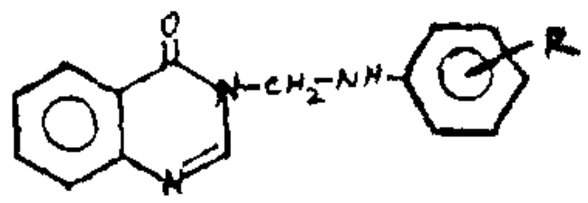
A SERIES of quinazolinone–4–N–Mannich bases have been synthesised from quinzolinone–4 and various primary aromatic amines in the presence of formaldehyde.

Compounds having an active hydrogen atom on nitrogen such as salicylamide[1], succinimide[2,3], phthalimide[4-6], 4-nitrophthalimide[7], isatins[8] and quinazolinones[9] have been reported to undergo ready Mannich condensations furnishing N–Mannich bases in good yields. Quinazolinone–4(I) may be considered a cyclic amide and as such the hydrogen atom attached at position 3 to nitrogen should be appreciably labile to participate in the Mannich condensation. It was therefore considered of interest to treat I with a few primary aromatic amines to furnish N–Mannich bases (II).

Fusion of anthranilic acid with formamide yielded I, which was then treated with formalin and primary amines in equimolar proportions. II thus obtained were characterised by means of elemental analyses and I.R. spectral data. As is evident from Table I this condensation reaction is possible with aromatic amines having electron donating as well as electron withdrawing substituents. That the Mannich reaction occurs at N–3 has already been established[9].



I



II

TABLE I

Quinazolinone-4-N-Mannich Bases(II)

Sl. No.	R	M.P. °C	Yield %	Molecular formula
1.	H	152	60	$C_{15}H_{13}N_3O$
2.	4–Me	135–36	55	$C_{16}H_{15}N_3O$
3.	4–OMe	160–61	65	$C_{16}H_{15}N_3O_2$
4.	4–Cl	186–87	55	$C_{15}H_{12}ClN_3O$
5.	3–Cl	166–67	50	$C_{15}H_{12}ClN_2O$
6.	2-OEt	148	55	$C_{17}H_{17}N_3O_2$
7.	4–Ph	174	60	$C_{21}H_{17}N_3O$
8.	4–COOH	215–17	60	$C_{16}H_{13}N_3O_3$
9.	4–COOMe	204	55	$C_{17}H_{15}N_3O_3$
10.	3–COOH	198–99	50	$C_{16}H_{13}N_3O_3$
11.	2–COOH	184–85	45	$C_{16}H_{13}N_3O_3$

All compounds gave satisfactory nitrogen analyses
I.R. (cm^{-1}) : 6 = 1675 (C = O), 3380 (NH), 1605 (C=N).
8 = 1678 (C=O, ring), 1700 (C=O, carboxylic), 3000 (OH, carboxylic), 3345 (NH), 1602 (C=N).

Experimental

Quinazolinone–4 was prepared according to published method[10,11].

Quinazolinone–4–N–Mannich bases(II) (*Table* I)

An intimate mixture of I (1·46 g; 0·01 mode), formalin (1·5 ml) and aniline (0·93 g.) in 20 ml of ethanol was warmed on a waterbath with stirring for 10 min and thereafter it was allowed to remain at room temp. overnight. The solid product thus separated was filtered and rectystallised from ethanol.

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ISOLATION AND CRYSTALLIZATION OF A METALLOPEPTIDE FROM THE OVARY OF SILKWORM, *BOMBYX MORI* L.

Introduction

While screening the ovary extract for aminotransferases[1], we came across a fraction which was held in Sephadex G–25 (Sigma Chemicals Co., which can exclude proteins of molecular weight above 5000 d) and reduces ferricyanide, a synthetic substrate for NADPH dehydrogenases[2]. Preliminary work showed that it contained iron and no sulphur. It was thought to be an iron metallopeptide of small molecular weight of either heme type or non-heme type. Its UV absorption (maximum at 294 nm) properties did not indicate the presence of heme. But most of the non-heme peptides are known to contain at least one to several atoms of inorganic sulphur per molecule, and both iron and sulphur atoms are found to be essential for the biological activity of the molecule[3]. However, there are a few non-heme peptides containing iron and are devoid of inorganic sulphur with biological activity[4], such as rubredoxin from *Clostridium*, which can substitute for ferredoxin in many of its biological reactions[5]. As this peptide differed from the known iron containing peptides, it was decided to undertake an investigation on this material. Hence in this paper preliminary findings on the isolation, crystallization and composition of this peptide are reported.

Methods

Six day old female pupae of silkworm *Bombyx mori* L. were collected. They were incubated at 25° C for three days. On the ninth day the pupae were sacrificed and the ovaries were collected in a beaker surrounded with ice. Ovaries were washed thoroughly with ice cold distilled water. The washed ovaries were homogenized in a Virtis homogenizer for 2 minutes as follows: first 40 seconds at 5000 rpm followed by 20,000 rpm for 40 seconds and 30,000 rpm for 40 more seconds. The homogenate was centrifuged at 3000 rpm for 90 min at 4° C in MSE refrigerated centrifuge. The supernatant was collected in a separating funnel and then mixed with one third of its volume of ice cold butanol and shaken thoroughly. The resulting emulsion was centrifuged at 3000 rpm for 1 hour at 4° C to remove the coagulated fat and lipoproteins into the butanol layer. The aqueous layer was stored in a refrigerator in an amber coloured bottle.

A column (100 × 2·2 cm) was packed with swollen Sephadex G–25–80 (Sigma Chemical Co.) and was equilibrated with 0·1 M NaCl. Ten ml of the aqueous layer, obtained after extraction with cold butanol was loaded on to the column, which was then eluted with 0·1 M NaCl containing 0·1% ascorbic acid. Eight ml fractions were collected. These fractions were screened in a Beckmann DU spectrophotometer at 280 nm. Fluorescence spectra of the peptide were obtained using Perkin-Elmer spectrofluorometer. The UV absorption spectra of the peptide fraction were taken at different pH levels.

For studying the amino acid composition, the peptide was hydrolysed with 6N hydrochloric acid for 20 h and the hydrolysate was suitably processed for paper chromatography. The estimation of iron was done using potassium thiocynate[6].

The peptide was digested with Pronase[7] for 24 h at 37° C in calcium acetate buffer at pH 7·8. The supernatant obtained after centrifugation of the peptide digest was deproteinized by keeping it in boiling water bath for 5 min. The clear supernatant after deprotenization was concentrated and used for paper chromatography of amino acids. The residue was suspended in water containing 0·2% cysteine and treated with 0·2% bipyridyl to remove the iron[8]. The residue left over, after complete removal of the iron by repeated treatment with bipyridyl in presence of cysteine, was found to be about 40–50% by weight of the solid peptide.

Paper chromatography of the amino acids was carried out first with butenol: acetic acid: water (4:1:5 V/V) and then with phenol-water (76:24 V/V) in an atmosphere of ammonia. The chromatograms were developed in 0·2% ninhydrin in acetone.

Results and Discussion

Fractionation of the ovary extract is given in Fig. I. The void volume of the column is 85 ml. The peptide is eluted between 170 and 215 ml and contains 1·4%