

(1705 cm^{-1}) and possibly carbon-carbon double bonds (1620 cm^{-1}) are present in each one of the polymeric materials investigated.

EXPERIMENTAL

Infrared spectra. The infrared spectra were recorded on a Perkin-Elmer, model 21, double beam infrared spectrophotometer. The sample for analysis (200 mg.) was ground in a mortar with anhydrous potassium bromide (1 g.) and the mixture (300 mg.) was pressed into a disk in a high vacuum under 18,000 p.s.i.

Preparation of humins from L-arabinose. Four 10-g. samples of L-arabinose ($[\alpha]_D^{25} + 104^\circ$) were respectively dissolved in 200 ml. of 5*N*, 10*N*, 15*N*, and 20*N* sulfuric acid contained in 1-liter flasks. The solutions were heated on the steam bath for a period of 12 hr. During this period a heavy brownish black precipitate formed. An equal volume of ice cold distilled water was then added to the contents of each flask and the precipitate removed by filtration on a fritted glass funnel. The precipitate was washed on the funnel with several liters of distilled water and then extracted with 1 liter of boiling ethanol, followed by 200 ml. of ether. The insoluble material was then dried at 80° in a high vacuum over anhydrous calcium chloride.

The ethanol extract was concentrated to dryness on the steam bath and then washed with ether and dried in a high vacuum over anhydrous calcium chloride. No differences were found in the carbon and hydrogen content of the polymers formed under the influence of the several concentrations of acid.

Anal. Found: C, 64.2 to 67.8; H, 4.2 to 4.9.

No analytical differences could be found between the ethanol-soluble and -insoluble material.

Anal. (ethanol-soluble). Found: C, 65.8 to 67.5; H, 4.1 to 4.9.

Preparation of humin from furfural. An amount of 0.96 g. of furfural was dissolved in 200 ml. of 10*N* sulfuric acid. The solution was heated on the steam bath in a flask equipped with a reflux condenser. The resulting polymeric material was then isolated in the same way as was the humin from L-arabinose (above).

Anal. (ethanol-soluble). C, 68.6; H, 4.6; (ethanol-insoluble), C, 68.4; H, 4.5.

Preparation of humin from furfural and crotonaldehyde. An amount of 0.95 g. of furfural together with 0.7 g. of crotonaldehyde was dissolved in 200 ml. of 10*N* sulfuric acid. The rest of the procedure was identical with the above.

Anal. (ethanol-soluble) C, 69.8; H, 6.6; (ethanol-insoluble), C, 70.4; H, 6.4.

Preparation of humin from a mixture of furfural, crotonaldehyde, acetaldehyde, and formaldehyde. A mixture consisting of 0.96 g. furfural, 0.7 g. of crotonaldehyde, 0.44 g. of acetaldehyde, and 0.30 g. of formaldehyde was dissolved in 200 ml. of 10*N* sulfuric acid.

Further treatment was the same as for furfural (above).

Anal. (ethanol-soluble). C, 63.0; H, 4.3; (ethanol-insoluble), C, 63.4; H, 4.7.

Acknowledgments. I should like to thank Mrs. P. P. Wheeler for the microanalyses, Mrs. N. F. Lyons for running the infrared spectra, and Mr. A. H. Johnstone for technical assistance in preparing the polymeric materials.

INDIAN HEAD, MD.

[CONTRIBUTION NO. 473 FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE, INC.]

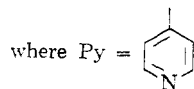
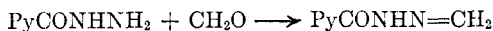
Synthetic Tuberculostats. XII. Structure of the Reaction Product of Isoniazid and Formaldehyde

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Received July 22, 1957

The compound prepared by the interaction of isoniazid with formaldehyde contains a triazane ring and is probably the monohydrate of hexahydro-1,3,5-triisonicotinamido-s-triazine.

During the course of an investigation into the tuberculostatic activity of isoniazid and its derivatives, an effort was made to synthesize 1-isonicotinyl-2-methylidenedihydrazine according to the following scheme:



The reaction was carried out by refluxing a mixture of isoniazid, aqueous formaldehyde (35%), and isopropyl alcohol. As the reaction proceeded, a fine white crystalline precipitate appeared which proved to be practically insoluble in all of the common solvents with the exception of dilute hydrochloric

acid and dilute sodium hydroxide. Since the anticipated methylidene derivative was expected to have solubility characteristics quite similar to those of the parent isoniazid, the marked insolubility of the product (hereinafter called Ro 2-4969) strongly suggested that it was not the desired compound at all. This was subsequently confirmed by elementary analysis.

In view of its marked tuberculostatic activity, it was decided to investigate the structure of the compound in some detail.

One obvious line of approach was to determine the molecular weight of the compound. Every attempt in this direction failed—principally because of its insolubility, although instability and possibly the presence of water of hydration were also contributory factors. Most of these attempts involved modifications of the Rast procedure with a variety

of solvents but an attempt was also made using the isothermal distillation method of Signer.¹

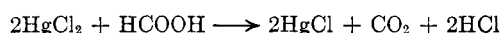
Failing a molecular weight determination, it seemed desirable to determine the ratio between methylene and hydrazide moieties in the molecule. Accordingly, Ro 2-4969 was oxidized with 30% H₂O₂ to isonicotinic and formic acids which were then quantitatively determined by titration (see Experimental for details).

In a series of experiments, the data listed in Table I were obtained. If a 1:1 ratio of methylene to isoniazid is assumed in a generalized structure such as [(PyCONHN)(CH₂)_x] then 2.86 grams and 1.43 grams of the compound should produce 0.38 mole and .019 mole of total acid respectively. This is in reasonable agreement with the experimental data (Table I).

TABLE I

Grams of Ro 2-4969 Used	Total Acid Formed, Moles
2.86	0.036
2.86	.0363
1.43	.0182
1.43	.0188
1.43	.0183

For further evidence, it was decided to determine whether half of the total acid formed by the peroxide oxidation was indeed formic acid. In order to distinguish between the formic acid and the isonicotinic acid formed in the oxidation mixture, advantage was taken of the fact that formic acid quantitatively reduces mercuric chloride to the insoluble mercurous chloride in accordance with the equation:



The titration mixture obtained after the peroxide oxidation and back-titration with standard hydrochloric acid was therefore treated with a large excess of mercuric chloride and was heated on a steam bath under a reflux condenser overnight (see Experimental for details). The precipitated mercurous chloride was washed, isolated, and dried. The data obtained in these experiments are listed in Table II.

TABLE II

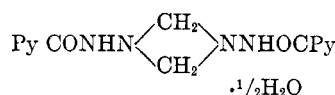
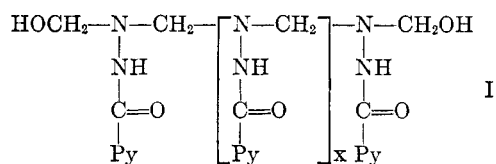
Wt. of Ro 2-4969 Oxidized, Gm.	Total Acid (Fd.), Moles	HCOOH (Calcd.), Moles	HCOOH (Fd.), Moles
1.43 ^a	—	—	0.00874
1.43	0.0182	0.0091	0.00877
1.43	0.0188	0.0094	0.00885

^a Total acid was not determined in this experiment.

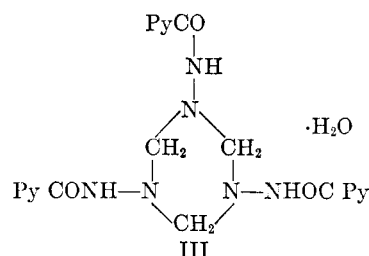
(1) R. Signer, *Ann.*, **478**, 246 (1930).

From these data it is apparent that each mole of Ro 2-4969 on peroxide oxidation yield 2 moles, or a multiple of 2 moles of acid, of which one half is formic acid and one half is isonicotinic acid. The ratio of methylene to isoniazid must be 1:1.

At this point in the study, sufficient data had been accumulated to permit the tentative selection and testing of several possible structures for Ro 2-4969, each of which constituted a reasonable surmise from the reactivity and the conditions of the reaction as well as from the known physical and chemical properties. These included a linear polymeric structure (I) and two cyclic structures—a dimeric hemihydrate (II) and a trimeric monohydrate or triazine structure (III).



II



III

Microchemical analyses of two different batches of Ro 2-4969 gave:

1. C, 54.4; H, 5.0
2. C, 54.6; H, 4.7

The linear structure corresponds quite satisfactorily to these findings in the interval between X = 2 and X = 5.

Calcd. where X = 2 mol.wgt. = 644 C = 54.0 H = 5.0
Calcd. where X = 5 mol. wgt. = 1091 C = 55.0 H = 4.9

A similarly satisfactory correspondence is obtained with the dimeric structure (II) which gives:

Calcd. for C₁₄H₁₆N₆O₂·½H₂O
mol. wt. = 307 C = 54.7 H = 4.9

and with the trimeric or triazine structure (III) which gives:

Calcd. for C₂₁H₂₁N₃O₃·1H₂O
mol. wt. = 465 C = 54.2 H = 4.9

When the structures were tested against the data obtained by the peroxide oxidation of Ro 2-4969 and the subsequent oxidation of the formic acid with mercuric chloride, it was found that the results calculated on the basis of the cyclic dimer

TABLE III

Structure	Mol. Wt.	Wt. of Sample-Gms.	Moles of Sample (a)	Calcd. Moles of Acid per Mole of Sample (b)	Total Acid		HCOOH	
					Calcd., moles (a) × (b)	Found, moles (Av.)	Calcd., moles	Found, moles (av.)
Linear	644	2.86	0.00444	9	0.0400	0.0362		
X = 2		1.43	.00222	9	.0200	.0184	0.0111	0.00879
X = 5	1091	2.86	.000262	15	.0392	.0362		
		1.43	.000131	15	.0197	.0184	.0105	.00879
Dimer (III)	307	2.86	.00932	4	.0373	.0326		
		1.43	.00466	4	.0186	.0184	.0093	.00879
Trimer (IV)	465	2.86	.00615	6	.0369	.0362		
		1.43	.00308	6	.0185	.0184	.0093	.00879

(II) and the trimer (III) structures were in close agreement with the experimental findings whereas those based on the "linear" (I) structure were widely divergent. This is illustrated in Table III.

There are other reasons for questioning the applicability of the linear structure. We have seen that to conform to the analytical findings, X must fall somewhere in the range between 2 and 5. Since all degrees of polymerization can be expected, X must perforce be an average figure and the average molecular weight must be somewhere between 644 and 1091. However, individual molecules could be expected to vary from 346 (where X = 0) to 1389 (where X = 7)—or even more. It is difficult to reconcile this picture with the fact that different batches of Ro 2-4969 made by different methods are indistinguishable and have reasonably sharp melting points in the vicinity of 172°.

The apparent homogeneity of Ro 2-4969 suggested the possibility of testing the validity of the linear polymeric structure by means of differential solubility determinations. If we assume the linear structure for Ro 2-4969 we can expect, with reasonable assurance, that any given sample of the substance would be made up of different sized molecules with varying solubilities in water. Such a sample should exhibit a marked decrease in solubility as the smaller, more soluble molecules are dissolved out and the larger, more insoluble ones are left behind.

Two procedures were used in establishing the solubility behavior of Ro 2-4969. In one of these, a series of samples of equal weight were suspended in varying volumes of water, shaken for equal lengths of time, and then filtered, dried, and weighed. The weights of the dissolved material were determined by difference. The data from these experiments are listed in Table IV.

In the other procedure, a weighed sample of the material was shaken with 100 cc. of water, filtered, dried, and weighed and the quantity in solution determined by difference. The dried precipitate was resuspended in 100 cc. of water and the procedure was repeated several times. The data obtained in these experiments are listed in Table V.

TABLE IV

SOLUBILITY OF Ro 2-4969 IN MILLIGRAMS PER 100 CC. WHEN SHAKEN WITH VARYING VOLUMES OF WATER

Vol. of H ₂ O, Cc.	Amount Dissolved (Mg.) in		Solubility, Mg./100 Cc.	
	5 hours	14 hours	5 hours	14 hours
100	22.2	25.1	22.2	25.1
500	—	100.4	—	20.
500	—	99.6	—	19.9
1000	190.1	189.8	19.	19.
1000	—	177.9	—	17.8
1000	—	187.1	—	18.7
2000	389	361.4	19.4	18.

It will be observed from Table IV that whereas the solubility is substantially constant, there is a small apparent decrease in solubility with increasing volume. A similar effect is noticeable in Table V where the solubility apparently decreases with repeated extraction of the same material. This is an artifact produced by the differences in particle (not molecular) size which exist within the sample. It is well known that the degree as well as the rate of solution is an inverse function of particle size. Proof of the fact that the observed phenomenon is due to particle rather than molecular size, is presented in Table V. In Experiment A, Table V, the solubility is seen to fall off approximately 1 mg. for each extraction. At the end of the 4th extraction, the material was very lightly ground in a mortar for a short time to increase the number of small particles, whereupon the solubility promptly increased in the 5th extraction and then proceeded to fall off again in the 6th extraction. Similarly, in Experiment B, the solubility increased when the material was ground after the 4th extraction.

Though the two remaining cyclic structures are equally satisfactory extrapolations of the data obtained so far, the trimer or triazine structure has several points in its favor. (1) The six membered ring of the trimer is inherently more stable than the four membered dimer ring. (2) The great insolubility of Ro 2-4969 bespeaks a larger molecule than the dimer. (3) The triazane ring of the trimer has

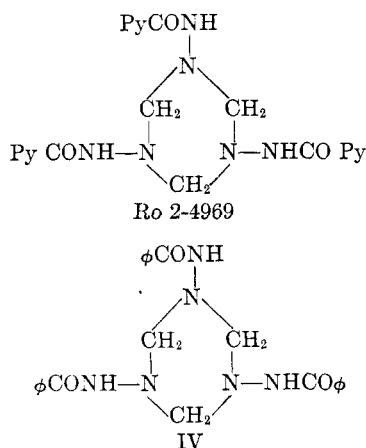
TABLE V
SOLUBILITY OF Ro 2-4969 IN SUCCESSIVE 100-CC. VOLUMES OF WATER

	Ro 2-4969, %	Time of Shaking	Solubility in Mg./100 Cc.					
			1st 100 cc.	2nd 100 cc.	3rd 100 cc.	4th 100 cc.	5th 100 cc.	6th 100 cc.
Expt. A	500 mg.	2 hrs.	19.7	18.8	17.6	16.4	20.2 ^a	18.8
Expt. B ^b	500 mg.	4 hrs.	24.1	24.5	20.2	18.4	20.0 ^a	18.7

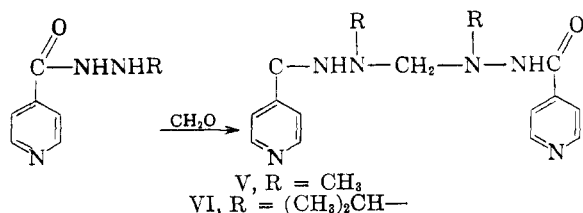
^a Substance ground a little at this point. ^b Substance ground at beginning of experiment.

been reported in the literature as the product of the reaction between formaldehyde and various substituted amines.

Since none of these points is sufficiently convincing to permit a choice to be made between the two structures, it was decided to study the infrared absorption curves of some pertinent compounds in the hope of obtaining conclusive evidence in favor of one of the structures. The compounds studied and some of their more significant absorption bands are listed in Table IV. From these data it can be seen that the only ones with absorption peaks in the regions 10.2–10.3 μ and 10.7–10.8 μ are Ro 2-4969, its benzene analog and the three compounds with known triazane structures. The only apparent common denominator is the probable presence of the triazane ring in Ro 2-4969 and its benzene analog IV.



To further test this concept, it was decided to react 1-isonicotinyl-2-methylhydrazine with formaldehyde in the hope of obtaining a methylene bis derivative of the type V.



Whether the product obtained actually has the hoped for structure is open to question, but that is of minor consideration.

The important fact is that the reaction could *not* produce a triazane ring and the product should therefore *not* absorb in the 10.2 and 10.7 regions. No such absorption was obtained. The analogous compound VI prepared from iproniazid and formaldehyde also failed to show absorption peaks at 10.2 and 10.7 μ .

Both of these compounds were obtained in the form of thermoplastic glasses or resins which could not be crystallized or purified. They are very soluble in water and in polar organic solvents and in this regard are very different from Ro 2-4969. These differences tend to support the infrared findings.

Since the triazane structure for Ro 2-4969 calls for a molecule of water of hydration in order to make it conform to the elementary analysis, a moisture determination was done by the Karl Fischer method.² The results of two determinations are as follows: (1) in methanol—found 3.8% moisture. (2) in methanol-pyridine—found 3.9% moisture. Calcd. for C₂₁H₂₁N₃O₃·1H₂O—3.9% moisture.

It is therefore established that Ro 2-4969 is hexahydro-1,3,5-triisonicotinamido-*s*-triazine monohydrate. By analogy the reaction product of benzoylhydrazine and formaldehyde is probably hexahydro-1,3,5-tribenzamido-*s*-triazine monohydrate.

EXPERIMENTAL

Peroxide oxidation of Ro 2-4969. To exactly 1.43 g. of Ro 2-4969 in a 400-cc. beaker fitted with a watch glass cover and a magnetic stirrer was added 6 g. (ca. 6 cc.) of 30% H₂O₂, exactly 25 cc. of 1N NaOH and 50 cc. of H₂O. The mixture, which vigorously evolved gas, was stirred and kept covered so that the entrained vapor condensed on the watch glass and dropped back into the beaker. When gas evolution had markedly diminished, the reaction mixture was brought to a boil and then allowed to cool spontaneously, with stirring. When gas evolution had ceased, the watch glass cover was carefully rinsed into the beaker with a little water and the mixture was then titrated with 1N HCl using a Beckmann pH meter. Neutralization required 6.85 cc. of 1N HCl.

25 cc. — vol. 1N HCl required = vol. of NaOH used

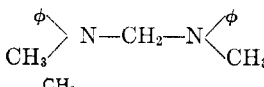
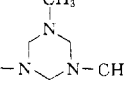
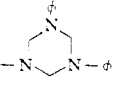
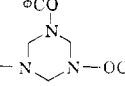
$\frac{\text{vol. of NaOH required}}{1,000} = \text{equivalents of acid formed}$

25 cc. — 6.85 cc. HCl = 18.15 cc. NaOH used
 $\frac{18.15}{1,000} = .01815 \text{ equivalents of acid}$

formed

(2) K. Fischer, *Angew. Chem.*, **48**, 394 (1935).

TABLE VI
 ABSORPTION PEAKS IN INFRARED REGION

Compound	Ref.	Wave Length in Microns						
		7.70	8.25- 8.35	8.55- 8.65	10.2- 10.3	10.7- 10.8	10.9- 11.0	11.65- 11.8
R-NHNH ₂	3	-	-	-	-	-	-	-
Ro 2-4969	E	+	+	+	+	+	+	+
RNHNHCH ₃	4	+	+	+	-	-	+	+
RN(CH ₃)NH ₂	5	+	-	-	-	-	-	-
RNHN(CH ₃) ₂	6	+	+	+	-	-	+	+
[RNHN=CH] ₂	5	+	+	+	-	-	+	-
Benzene analog of Ro 2-4969	E	+	+	+	+	+	+	+
R-N-N(CH ₃) ₂ CH ₂ φ	5	-	-	-	-	-	+	+
φCONHNH ₂	3	-	-	-	-	-	+	-
(CH ₃) ₂ N-CH ₂ -N(CH ₃) ₂	7	-	+	+	-	-	-	-
	8	-	+	+	-	-	-	-
	7	+	+	+	+	-	+	+
	9	-	+	+	+	+	+	-
	10	+	+	+	+	+	+	-

R = PyCO-. E—See Experimental. - equals no peak. + equals peak.

Formic acid determination. To a titrated mixture from the peroxide oxidation transferred to a round bottom flask fitted with a condenser was added 15 g. of mercuric chloride. The clear mixture was heated on a steam bath overnight to give a lustrous precipitate of mercurous chloride. The mixture was filtered hot and the precipitate was washed successively with water, methanol, and ether and finally dried. The precipitated mercurous chloride weighed 4.132 g.

4.132 g. HgCl \equiv .403 g. HCOOH

.403 g. HCOOH \equiv .00877 moles HCOOH

Syntheses. Ro 2-4969 (Hexahydro-1,3,5-triisonicotinamido-s-triazine monohydrate). A mixture of 30 g. of isonicotinylhydrazine, 30 cc. of 35% aqueous formaldehyde, and 200 cc. of 2-propanol is heated under reflux. A fine white crystal-

(3) H. H. Fox and J. T. Gibas, *J. Org. Chem.*, **17**, 1653 (1952).

(4) H. H. Fox and J. T. Gibas, *J. Org. Chem.*, **18**, 994 (1953).

(5) H. H. Fox and J. T. Gibas, *J. Org. Chem.*, **21**, 356 (1956).

(6) H. H. Fox and J. T. Gibas, *J. Org. Chem.*, **20**, 60 (1955).

(7) L. Henry, *Bull. Acad. roy. de Belgique* [3] **26**, 200 (1893).

(8) J. v. Braun, *Ber.*, **41**, 2147 (1908).

(9) G. Pulvermacher and W. Loeb, *Ber.*, **25**, 2765 (1892).

(10) P. Duden and M. Scharff, *Ann.*, **288**, 251 (1895).

line precipitate begins to appear almost before the isonicotinylhydrazine is completely dissolved. After refluxing for about 0.5 hr., most of the solvent is removed to yield 25 g. of product; m.p. 171.5–173.5° corr.

Anal. Calcd. for C₂₁H₂₁N₉O₃·1H₂O: C, 54.2; H, 4.9. Found: C, 54.4; H, 5.0.

The same product can be obtained by substituting paraformaldehyde for aqueous formaldehyde, or water for 2-propanol as a solvent.

Benzene analog of Ro 2-4969 (hexahydro-1,3,5-tribenzamido-s-triazine monohydrate). Add 15 cc. of 38% aqueous formaldehyde to a solution of 20 g. of benzoylhydrazine in 400 cc. of hot 2-propanol. The mixture is warmed on a steam bath for about 0.5 hr. and the pure product which precipitates is collected. The product is obtained in the form of small white needles which melt with decomposition and prior softening at 160–163° corr. and which are insoluble in all common solvents including dilute hydrochloric acid and dilute sodium hydroxide solution.

Anal. Calcd. for C₂₄H₂₄N₉O₃·H₂O: C, 62.3; H, 5.6. Found: C, 62.6; H, 6.1.

Acknowledgment. Thanks are due Mr. B. Pecherer for valuable suggestions and to Mr. D. Wagner for technical assistance during the course of the infrared studies. The author also acknowledges his indebtedness to Dr. A. Steyermark and his staff for the microanalysis.

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