[CONTRIBUTION FROM THE WILLIAM H. NICHOLS LABORATORY, NEW YORK UNIVERSITY]

Condensation Reactions of Cinchoninaldehyde and Quinaldaldehyde with Some Heterocyclic Methylene Compounds. I

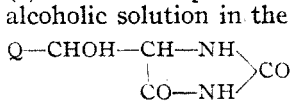
BY ARTHUR P. PHILLIPS[1]

Previous work[2] with cinchoninaldehyde (I) and quinaldaldehyde (II) has been continued in the condensation of these aldehydes with a series of heterocyclic, reactive methylene compounds. The heterocyclic compounds used as starting materials and included in this paper are: hydantoin, 2-thiohydantoin, 1-benzoyl-2-thiohydantoin, 1-acetyl-2-thiohydantoin, rhodanine, pseudothiohydantoin, N^2-phenylpseudothiohydantoin, N^2,3-diphenylpseudothiohydantoin, 5-methylpseudothiohydantoin, and 5-methyl-N^2,3-diphenylpseudothiohydantoin. Several other heterocyclic compounds have been condensed with the quinoline aldehydes and the results will be published at a later date.

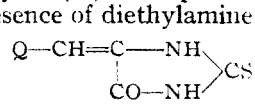
The literature reports condensations of most of the reactive methylene compounds listed above with a great variety of aromatic aldehydes under various reaction conditions. The method most commonly described makes use of glacial acetic acid as the reaction medium, in the presence of anhydrous sodium acetate, with or without acetic anhydride.

The present series of condensations has been carried out by mixing the reactants in alcohol or aqueous alcohol with a few drops of diethylamine as the condensing agent, except in a few cases where reaction occurred so rapidly that no catalyst seemed necessary. In a few cases the product has been made in the acetic acid medium as well.

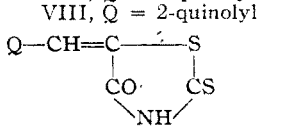
Hydantoin[3] condensed with cinchoninaldehyde (I) and with quinaldaldehyde (II) in aqueous alcoholic solution in the presence of diethylamine



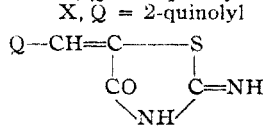
III, Q = 4-quinolyl
VIII, Q = 2-quinolyl



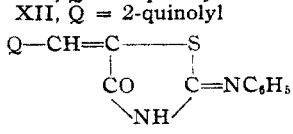
IX, Q = 4-quinolyl
X, Q = 2-quinolyl



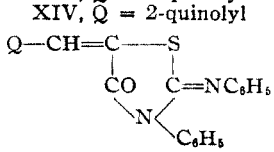
XI, Q = 4-quinolyl
XII, Q = 2-quinolyl



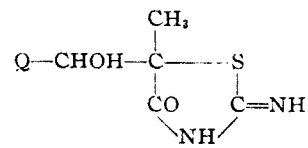
XIII, Q = 4-quinolyl
XIV, Q = 2-quinolyl



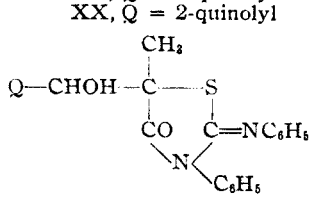
XV, Q = 4-quinolyl
XVI, Q = 2-quinolyl



XVII, Q = 4-quinolyl
XVIII, Q = 2-quinolyl



XIX, Q = 4-quinolyl
XX, Q = 2-quinolyl



XXI, Q = 4-quinolyl
XXII, Q = 2-quinolyl

to give aldol products (III and VIII). These results were especially of interest because no report was found in the literature of any aromatic aldehyde condensing with hydantoin to give an aldol compound, the usual product being of the benzylidene hydantoin type.

The aldol compounds III and VIII were dried to constant weight at elevated temperature (110°) in vacuum (6 mm.) over phosphorus pentoxide, after which analytical results indicated the structures shown. Further work concerning the structure of the aldol products from hydantoin was carried out with cinchoninaldehyde as this was more readily available.

For an indication as to whether the point of attachment of the hydroxymethyl (quinolyl) group was at the 5-position of the hydantoin ring (as shown), three 5,5-disubstituted hydantoins (5,5-dimethylhydantoin,[4] 5,5-diphenylhydantoin,[5] and the spirohydantoin[6] derived from cyclopentanone by the Bucherer synthesis) were made and each was mixed with cinchoninaldehyde and submitted to the same reaction conditions used with hydantoin itself. In each case, however, there was no evidence of reaction and upon working up the reaction mixture a nearly complete recovery of the 5,5-disubstituted hydantoin was realized. Thus it would seem probable that in the successful condensation with hydantoin itself the active methylene group, position 5, is involved in the reaction.

More direct evidence as to the structure of III was obtained as outlined in the Chart below.

Thus I with hydantoin under the conditions of the Perkin condensation gave the unsaturated product IV (33% yield). Compound IV was also obtained, in 20% yield, from the aldol III by re-

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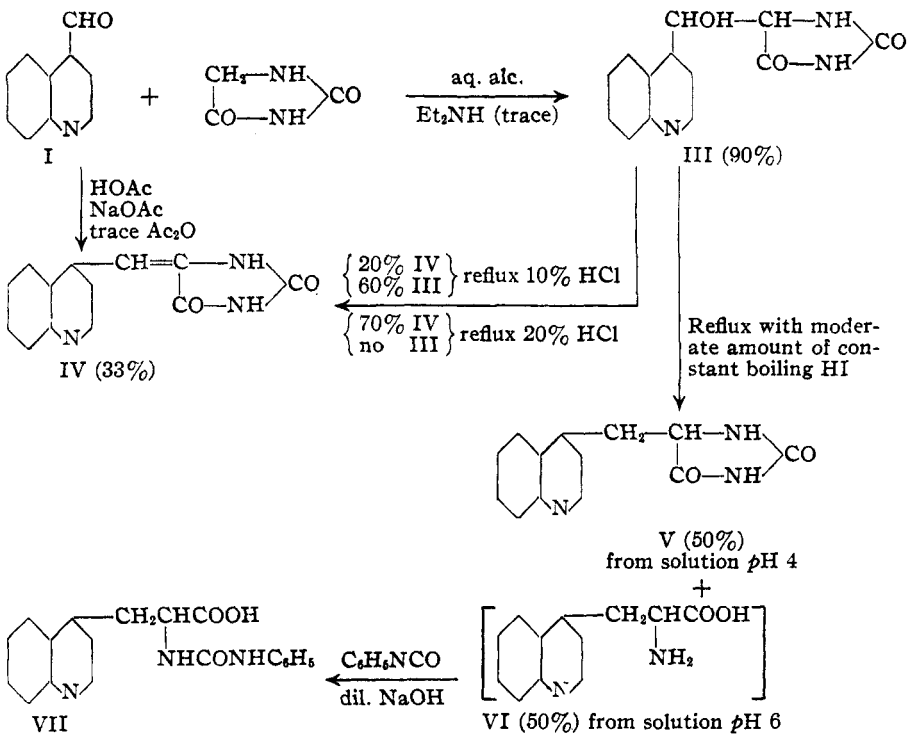
(2) Kwartler and Lindwall, THIS JOURNAL, **59**, 524 (1937); Kaplan and Lindwall, *ibid.*, **65**, 927 (1943).

(3) Wagner and Simons, *J. Chem. Educ.*, **13**, 266 (1936).

(4) Bucherer, *J. prakt. Chem.*, **141**, 5 (1935).

(5) Biltz, *Ber.*, **41**, 1391 (1908).

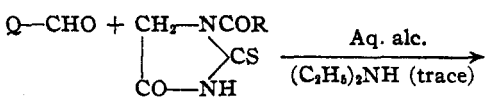
(6) Henze, THIS JOURNAL, **64**, 522 (1942).



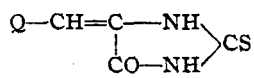
fluxing with 10% hydrochloric acid, while 60% of III was recovered unchanged. Refluxing III with 20% hydrochloric acid gave 70% of IV and no III was recovered. Compound III on refluxing with varying amounts of constant boiling hydriodic acid[7] (sp. gr. 1.7) gave products V and VI. A small proportion of hydriodic acid with brief reflux period gave almost entirely V, moderate amounts of hydriodic acid refluxed about the same period gave about equal amounts of the reduced product V and its hydrolysis product VI, while a large volume of hydriodic acid and extended reflux period gave only VI. Compound VI was not readily obtained analytically pure and thus was converted by phenyl isocyanate in weakly alkaline solution to the phenylurea derivative VII. With ninhydrin VI gave a strong positive test (characteristic of α-amino acids) different in quality from that obtained with glycine, while III, IV, V and hydantoin gave no test with ninhydrin.

Compounds III, IV, and V were soluble in aqueous alkali and were precipitated unchanged by aqueous mineral acid at *p*H 3.5–4.5. They were recrystallized from aqueous alcohol.

2-Thiohydantoin[8] condensed with cinchoninaldehyde (I) and with quinaldaldehyde (II) to give in each case unsaturated products, IX and X, respectively. IX and X were also formed by the condensation of the quinoline aldehydes with either 1-benzoyl-2-thiohydantoin or 1-acetyl-2-thiohydantoin under the same reaction conditions:



Q = 4-quinolyl or 2-quinolyl
R = CH_3 or C_6H_5



Rhodanine[9] combined so rapidly in aqueous alcohol solution with each of the two aldehydes employed that no diethylamine was added as condensing agent. The products, XI and XII, were both of the "benzylidene" type.

Pseudothiohydantoin,[10] N^2-phenylpseudothiohydantoin[11] and N^2,3-diphenylpseudothiohydantoin[12] reacted with the quinoline aldehydes under the usual conditions to give in every instance an unsaturated product (XIII, XIV, XV, XVI, XVII, XVIII).

Products of an aldol-like nature were desired and, since most of those obtained were of the unsaturated type, 5-methylpseudothiohydantoin[13] and 5-methyl-N^2,3-diphenylpseudothiohydantoin[13] were prepared. In these compounds one of the hydrogens of the active methylene group has been replaced by the immobile methyl radical, and thus, if still active enough to condense, they are likely to form aldol products, for they cannot

(7) Boyd and Robson, *Biochem. J.*, **29**, 546 (1935).
(8) Johnson and Nicolet, THIS JOURNAL, **33**, 1973 (1911).
(9) Nencki, *J. prakt. Chem.*, [2] **16**, 1 (1877).
(10) Volhard, *Ann.*, **166**, 383 (1873); *J. prakt. Chem.*, [2] **9**, 7 (1874).
(11) Paul Meyer, *Ber.*, **14**, 1661 (1881).
(12) Lange, *ibid.*, **12**, 596 (1879); Markley and Reid, THIS JOURNAL, **52**, 2137 (1930).
(13) Dixon, *J. Chem. Soc.*, **63**, 819 (1893); **71**, 617 (1897).

TABLE I

CONDENSATION REACTIONS OF QUINOLINE ALDEHYDES: A. CINCHONINALDEHYDE PRODUCTS

Reactive methylene	Product[a]	Reflux time, hr.	Yield, %	M. p., °C.[b]	Empirical formula	Analyses, % Calculated C	Calculated H	Calculated N	Found C	Found H	Found N	
Hydantoin	III[c]	1.5	81	257–259 dec.	$C_{13}H_{11}O_2N_3$	60.7	4.32	16.3	60.9 60.8	4.09 4.34	16.2 16.5	16.4
Hydantoin	IV[c,d]	8.0	33	335–336	$C_{13}H_9O_2N_3$	65.2	3.81	17.6	65.0	3.65	17.5	
2-Thiohydantoin	IX[e,f]	4.0	86	>320	$C_{13}H_9ON_3S$	61.1	3.56	16.5	61.1	3.66	16.5	16.4
Rhodanine	XI[f,g]	2.0	87	293–295 dec.	$C_{13}H_9ON_2S_2$	57.4	2.96	10.3	57.5	3.41	10.5	
Pseudothiohydantoin	XIII[f]	1.0	73	294–296 dec.	$C_{13}H_9ON_3S$	61.1	3.56	16.5	60.8	3.58	16.3	16.6
N^1-Phenylpseudothiohydantoin	XV[h]	4.0	76	295–296 dec.	$C_{19}H_{13}ON_3S$			12.7			12.6	
N^1,3-Diphenylpseudothiohydantoin	XVII[i,j,m]	4.0	70	229–230	$C_{25}H_{17}ON_3S$	73.7	4.18	10.3	73.9	4.37	10.4	10.5
5-Methylpseudothiohydantoin	XIX[e]	5.0	28	196–198	$C_{14}H_{13}O_2N_3S$	58.6	4.53	14.6	59.0	4.79	14.4	
5-Methyl-N^1,3-diphenylpseudothiohydantoin	XXI[e]	5.0	71	194–195	$C_{26}H_{21}O_2N_3S$	71.0	4.78	9.57	71.3	4.66	9.35	9.47

B. QUINALDALDEHYDE PRODUCTS

Hydantoin	VIII[c]	1.0	60	201–203 dec.	$C_{13}H_{11}O_2N_3$	60.7	4.32	16.3	61.0	3.98	16.1	16.2
2-Thiohydantoin	X[f,k]	2.0	89	296–297 dec.	$C_{13}H_9ON_3S$			16.5			16.4	16.5
Rhodanine	XII[g,l]	0.5	86	273–275 dec.	$C_{13}H_9ON_2S_2$	57.3	2.94	10.3	57.4	3.19	10.3	
Pseudothiohydantoin	XIV[e,j]	2.0	80	285 dec.	$C_{13}H_9ON_3S$			16.5			16.6	16.6
N^1-Phenylpseudothiohydantoin	XVI[k,m]	2.0	61	266–267 dec.	$C_{19}H_{13}ON_3S$			12.7			12.7	12.8
N^1-3-Diphenylpseudothiohydantoin	XVIII[j]	3.0	84	209–210	$C_{25}H_{17}ON_3S$	73.7	4.18	10.3	73.9	4.37	10.1	
5-Methylpseudothiohydantoin	XX[e]	2.0	35	177–178 dec.	$C_{14}H_{13}O_2N_3S$	58.6	4.53	14.6	58.8	4.82	14.4	
5-Methyl-N^1,3-di-phenylpseudothiohydantoin	XXII[e]	3.0	85	188–190 dec.	$C_{26}H_{21}O_2N_3S$	71.0	4.78	9.57	71.5	4.93	9.42	9.68

[a] Products made by Method A unless otherwise stated, all were obtained as yellow needles except the aldols and compound IV which were had as white needles. [b] All melting points are uncorrected. [c] Recrystallized from aqueous alcohol. [d] Prepared by Method C; also obtained from III by refluxing with hydrochloric acid. [e] IX was also obtained from either 1-benzoyl or 1-acetyl-2-thiohydantoin under the same conditions and in the same yields. [f] Recrystallized from pyridine. [g] Made by Method B. [h] Recrystallized from aqueous pyridine. [i] Compound XVII was also obtained in 22% yield by Method C. [j] Recrystallized from aqueous acetic acid. [k] Compound X was also obtained from 1-benzoyl or 1-acetyl-2-thiohydantoin under the same conditions and in the same yields. [l] Recrystallized from glacial acetic acid. [m] Recrystallized from alcohol.

split out the elements of water forming an unsaturated product. These two, on condensation with cinchoninaldehyde and quinaldaldehyde, produced aldol-like compounds (XIX, XX, XXI, XXII).

In the double series of compounds made, where similar products were obtained from both cinchoninaldehyde and quinaldaldehyde, it was interesting to note certain generalizations:

(1) Quinaldaldehyde appears to be slightly more reactive than cinchoninaldehyde as evidenced by somewhat greater yield obtained from it in most of the condensations, in spite of the fact that the cinchoninaldehyde products, less soluble, probably were precipitated more completely from their reaction mixtures.

(2) Consistently lower melting points, greater solubility and ease of recrystallization were manifested by the derivatives of quinaldaldehyde as compared with the cinchoninaldehyde products.

Experimental

Condensations.—Nearly all the condensation reactions were carried out in the same general manner, but in a few instances minor variations were employed. The principal method and the variations are outlined below.

Method A.—The quinoline aldehyde (0.01 mole) to be condensed was dissolved in slightly more than the minimum amount of hot alcohol. The reactive methylene compound (0.01 mole) was separately dissolved in a little more than the minimum amount of either hot alcohol or hot water, always using that solvent in which the starting compound was the more soluble. These two solutions were filtered hot, separately, then combined and mixed well by swirling. A few drops (3 or 4) of diethylamine was added to the mixture as a condensing agent and the solution was refluxed for a period of time varying from one to six hours, depending upon the visible manifestations of reactions, such as precipitation of product, which frequently occurred within a few minutes. Even when precipitation was immediate, refluxing was continued for some time to insure completion of the reaction. Finally, the reaction mixture was cooled, the precipitated product filtered by suction, washed well with water, then with alcohol, dried, recrystallized and prepared for analysis.

Method B.—In some cases the product was formed and came down so rapidly that no catalyst (diethylamine) was added, but refluxing was permitted for about an hour to complete the reaction.

Method C.—The quinoline aldehyde (0.01 mole) and reactive methylene compound (0.01 mole) were mixed in a solution of 4–5 cc. of glacial acetic acid and 2 g. of fused sodium acetate. The mixture was heated on the steam-bath for a period of from two to eight hours, then was cooled and poured into cold water (25–30 cc.). The precipitated product was collected by filtration and recrystallized.

Drying Experiments.—The aldol products were dried to constant weight at 110° in vacuum (6 mm.) over phosphorus pentoxide before analysis.

Structure of Compound III

Compound III.—From cinchoninaldehyde and hydantoin: A solution of 1.6 g. of I in 15 cc. of hot ethyl alcohol was mixed with a solution of 1.0 g. of hydantoin in 10 cc. of hot water. To this was added 3 drops of diethylamine

and the mixture was refluxed for two hours. Precipitation of product started within five minutes. After two hours the mixture was cooled, filtered, and the product washed with alcohol; recrystallized from aqueous alcohol as white needles, m. p. 258–259°; yield 2.4 g. (90%).

Anal. Calcd. for $C_{13}H_{11}O_3N_3$: C, 60.7; H, 4.32; N, 16.3. Found: C, 60.8; H, 4.34; N, 16.2, 16.4.

Compound III was soluble in dilute sodium hydroxide solution and was precipitated unchanged upon addition of dilute hydrochloric acid to *p*H 3.5–4.5; m. p. 257–258°; a mixed melting point of this product with the product originally obtained from the condensation was 257–258°.

5,5-Dimethylhydantoin and I.—Compound I (1.5 g.) and 5,5-dimethylhydantoin (1.3 g.) were mixed and dissolved in 50 cc. of 50% alcohol, 3 drops of diethylamine was added and the mixture was refluxed four hours on the steam-bath. Since after cooling no precipitate formed, the reaction mixture was evaporated almost to dryness on the steam-bath and extracted 8 times with ether. Evaporation of the ether extracts to a small volume gave 1 g. of white crystals; m. p. 174–175°; recrystallized from water, m. p. 174–175°. The original 5,5-dimethylhydantoin melted at 174–175° and a mixture of the original and recovered material melted at 174–175°. The 1 g. recovered represents 77% of the dimethylhydantoin used. This particular substance was especially hard to recover because of its great solubility in water and slight solubility in ether.

5,5-Diphenylhydantoin and I.—Compound I (1.6 g.) and 5,5-diphenylhydantoin (2.5 g.) were mixed and dissolved in 125 cc. of hot absolute alcohol, 3 drops of diethylamine was added and the mixture was refluxed for five hours on the steam-bath. Cooling gave no precipitate, but upon evaporation of the reaction mixture to about 25 cc. volume, followed by cooling, there was obtained 2.5 g. (100%) of white crystals; m. p. 293–294°; recrystallized from absolute alcohol gave 2.5 g., m. p. 293–294°. The original diphenylhydantoin melted at 293–294° and a mixture of the original and recovered samples melted at 293–294°.

Cyclopentanone Spirohydantoin and I.—Compound I (1.6 g.) and the spirohydantoin (1.6 g.) obtained from cyclopentanone were mixed and dissolved in 50 cc. of 50% alcohol, 3 drops of diethylamine was added, and the mixture was refluxed for three hours on the steam-bath. No product was obtained on cooling so the mixture was evaporated to dryness and the residue was extracted 8 times with ether. Evaporation of the ether extracts gave 1.5 g. (90%) of white crystals; m. p. 204–205°, recrystallized from water, gave 1.4 g., m. p. 204–205°. The original spirohydantoin melted at 204–205° and a mixture of the original and recovered material melted at 204–205°.

It would seem from the above four experiments that the successful condensation with hydantoin could only have taken place at the 5-position of the hydantoin ring.

Compound IV: From I and Hydantoin.—I (4.5 g.) and hydantoin (3.0 g.) were mixed and dissolved in a solution of 15 cc. of glacial acetic acid and 5 g. of fused sodium acetate. Three drops of acetic anhydride was added and the mixture was heated eight hours on the steam-bath. A precipitate separated from the hot solution. The reaction mixture was cooled, filtered, and the solid washed well with acetic acid, much cold water, and absolute alcohol. The white solid was boiled up with absolute alcohol and filtered hot; yield, 2.4 g. (33%) white solid; m. p. 327–332°; recrystallized from aqueous alcohol; m. p. 335–336°.

Anal. Calcd. for $C_{13}H_9O_2N_3$: C, 65.2; H, 3.81; N, 17.6. Found: C, 65.0; H, 3.65; N, 17.5.

This product was soluble in dilute sodium hydroxide and was precipitated unchanged by the addition of dilute hydrochloric acid; white solid, m. p. 335–336°.

Compound IV: From III.—Compound III (1 g.) was dissolved in 25 cc. of 10% hydrochloric acid and the solution was refluxed for three hours, then cooled in the ice box. From the chilled solution there crystallized a yellow-white solid which was filtered off and washed with absolute alcohol and ether. This product was dissolved in dilute sodium hydroxide and precipitated with dilute hydrochloric acid; yield, 0.60 g. (60%); m. p. 257–258°. The substance was recrystallized from dilute alcohol; m. p. 258–259° (60% unchanged III).

Anal. Calcd. for $C_{13}H_{11}O_3N_3$: C, 60.7; H, 4.32; N, 16.3. Found: C, 60.9; H, 4.35; N, 16.0.

The mother liquors from the original precipitate were neutralized to *p*H 7 with dilute sodium hydroxide and a small amount of white solid was obtained; 0.20 g. (20%); m. p. 330–335°. This substance was purified by reprecipitation from a dilute alkaline solution with dilute acid; followed by recrystallization from dilute alcohol; m. p. 335–336° (20% of IV).

Anal. Calcd. for $C_{13}H_9O_2N_3$: N, 17.6. Found: N, 17.3.

Compound III (1.5 g.) and 40 cc. of 20% hydrochloric acid were mixed and refluxed for five hours. On cooling 1.0 g. (70%) of white crystals separated. These crystals were dissolved in dilute alkali and reprecipitated with dilute acid, then recrystallized from dilute alcohol; white needles; m. p. 335–336° (70% of IV).

Anal. Calcd. for $C_{13}H_9O_2N_3$: C, 65.2; H, 3.81; N, 17.6. Found: C, 65.0; H, 3.99; N, 17.6.

Compound V: From III.—Compound III (1 g.) was refluxed for four hours with 15 cc. of constant boiling hydriodic acid (sp. gr. 1.7). The solution was evaporated to dryness in vacuum, the residue was dissolved in water and brought to *p*H 5 with dilute alkali, precipitating a white solid; 0.8 g. (80%); m. p. 305–310° (dec.). This crystallized from aqueous alcohol as white, felted needles; m. p. 307–310° (dec.).

Anal. Calcd. for $C_{13}H_{11}O_2N_3$: C, 64.7; H, 4.60; N, 17.4. Found: C, 64.5; H, 4.40; N, 17.3, 17.4.

Compound VI: From III.—Compound III (3 g.) was refluxed for six hours with 100 cc. of constant boiling hydriodic acid (sp. gr. 1.7) and the solution was then evaporated to dryness in vacuum. The residue was taken up in water and brought to *p*H 6 with dilute alkali, when a white solid precipitated; crude yield 2.1 g. (80%); m. p. 215–230° (slow dec.). Compound VI was soluble in a small volume of hot water, but neither cooling nor addition of absolute alcohol gave a precipitate, although VI was insoluble in absolute alcohol. To recover VI the aqueous solution had to be evaporated to dryness in vacuum.

Other properties of VI were: (1) It gave a deep purple-reddish color (plum colored) when heated a few minutes in aqueous solution with ninhydrin. (Glycine under the same conditions gave a deep purple-bluish color, while hydantoin, and compounds III, IV, and V gave no color reaction with ninhydrin.) (2) VI heated with aqueous ferric chloride solution gave a reddish-brown colored solution of the same color and intensity as that obtained under similar conditions with glycine and the ferric chloride solution. (3) VI, though soluble in warm water, was much less soluble than was glycine.

It was difficult to obtain a sample of VI analytically pure, because so far no adequate method of crystallizing it has been devised, thus a portion of the substance was converted to the phenylurea derivative VII.

Compound VII: From VI.—A sample of the crude VI was dissolved in the calculated amount of 0.1 *N* sodium hydroxide, the equivalent amount of phenyl isocyanate was added, and the mixture was shaken for a few minutes. It was filtered, next, to remove any carbanilide which may have formed and dilute hydrochloric acid was added to the clear filtrate to *p*H 4. A white solid precipitated and this was filtered by suction, washed well with water, absolute alcohol, and ether; recrystallized with difficulty from aqueous alcohol; m. p. 225–226° (dec.).

Anal. Calcd. for $C_{19}H_{17}O_3N_3$: N, 12.5. Found: N, 12.3.

More work is being done with compound VI and its derivatives and it is hoped that further results along this line may be published later.

Summary

Cinchoninaldehyde and quinaldaldehyde have

been condensed with a varied series of heterocyclic compounds.

2-Thiohydantoin, 1-benzoyl-2-thiohydantoin, 1-acetyl-2-thiohydantoin, rhodanine, pseudothiohydantoin, N^2-phenylpseudothiohydantoin, and N^2,-3-diphenylpseudothiohydantoin on condensation with the quinoline aldehydes gave products of the unsaturated "benzylidene" type in every instance. In the case of the 1-benzoyl and 1-acetyl-2-thiohydantoins, condensation was accompanied by simultaneous loss of the acyl group.

Hydantoin, 5-methylpseudothiohydantoin and 5 - methyl - N^2,3 - diphenylpseudothiohydantoin combined with the quinoline aldehydes producing aldol-like compounds in each case.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

The Relation between the Structure of Heparin and its Anticoagulant Activity

BY M. L. WOLFROM AND W. H. MCNEELY[1]

In a previous publication[2] from this Laboratory, it was reported that the crystalline barium acid heparinate (from beef) of Charles and Scott[3] lost its biological activity on repeated crystallization from dilute acetic acid. Since this might indicate that the barium acid heparinate was merely a carrier for an active impurity, this inactivation was subjected to further investigation, the results of which are herewith reported.

The anticoagulant activities were determined in this Laboratory essentially according to the Foster[4] modification of the procedure of Reinert and Winterstein[5] and are expressed as Roche anticoagulant units per milligram (Roche ACU per mg.). It was found more convenient and more conducive to uniform results, to prepare a mixed sample of citrated beef plasma from a number of animals and to store the frozen plasma in small bottles for use as needed.

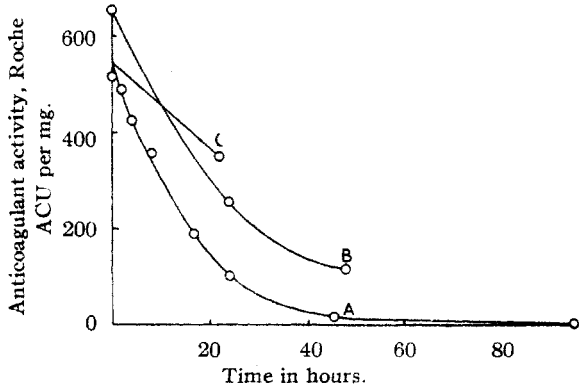

Fig. 1.—Inactivation of heparin salts (c 2, 68 ± 2°) by mild acidity. A, barium acid heparinate in 11% acetic acid (*cf.* Table I); B, sodium heparinate in 11% acetic acid; C, barium acid heparinate in water.

Unless otherwise noted, the barium acid heparinate used in the present work was the crystalline material prepared through the benzidine salt and the sodium heparinate used was the amorphous material prepared from this crystalline barium acid heparinate.

Curve A of Figs. 1 and 2 shows the rate of inactivation of a 2% solution of barium acid heparinate in 11% acetic acid solution at 68 ± 2°. The activities at each point were determined on the whole solution. Forty-eight hours of such treatment led to an essentially inactive solution from which there could be obtained with some difficulty, in 55% yield, a crystalline product similar in appearance to the original crystalline barium acid heparinate. These crystals were, however, considerably more soluble in dilute aqueous acetic acid (containing barium acetate) than the active crystals. In one experiment, crystallization was effected at the eight and twenty-two hour points of Curve A. A yield of 47 and 30%, respectively, of well-formed crystals was obtained. No significant differences in the activities, expressed as

TABLE I

INACTIVATION OF BARIUM ACID HEPARINATE (c 2, 68 ± 2°) IN 11% ACETIC ACID

Time, hr.	Solution activity, Roche ACU per mg.	Precipitate activity, Roche ACU per mg.	Precipitation yield, %
0		515	
2.1	490		
4.0	425		
8.0	355		
16.7	190		
24		95	86
24		115	94
24	100		
24	95		
45.5	15		
45.5		15	
95		3	
95	0		

(1) Hoffmann-La Roche Postdoctoral Fellow of The Ohio State University Research Foundation.

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