[CONTRIBUTION FROM THE MAYURBHANJ CHEMICAL LABORATORY, RAVENSHAW COLLEGE]

Preparation of 2-Substituted-4-methyl-5-carbethoxythiazoles. Condensation of Ethyl Acetoacetate with Substituted Thioureas

BY M. K. ROUT AND H. K. PUJARI

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A number of 2-substituted 4-methyl-5-carbethoxythiazoles and their mercurated derivatives have been prepared for evaluation of their biological activities.

In view of the usefulness of thiazole compounds[1-4] in therapy and for other effects, it was considered worthwhile to initiate investigation of a convenient method for the preparation of 2-aminothiazoles and their N-substituted derivatives.

For the synthesis of these compounds, several methods[5-7] have been used but the method suggested here (a modification of that of Dodson and King[8]) is more convenient and offers the advantage that different substituents, including even hetero rings, can be introduced easily at different positions of the thiazole nucleus, depending on the nature of ketone or ketonic ester taken as the starting material.

The observation of Dodson, Hlavacek and King[8,9] that ketones and ketonic esters react with thioureas in presence of halogens to give 2-aminothiazoles has been examined here as a preparative method for secondary thiazolylamines by condensing ethyl acetoacetate with substituted thioureas in the presence of iodine. The general experimental procedure adopted for the preparation of thiazoles has been illustrated by the synthesis of 2-phenylamino-4-methyl-5-carbethoxythiazole.

The thiazole bases have all been mercurated. On mercuration, the acetoxymercuri group enters the phenyl nucleus, since it has been found that 2-amino-4-methyl-5-carbethoxythiazole (in which the aromatic part is absent) does not undergo mercuration under the experimental conditions adopted in the present investigation. On the basis of earlier observations on the mercuration of aromatic amines,[10] it has been assumed that the acetoxymercuri group enters the para position (with respect to –NH group) of the aryl nucleus in the thiazole base and if the para position is blocked, ortho substitution occurs.

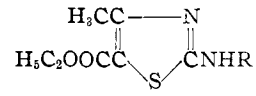
Experimental

Substituted Thioureas.—The phenyl- and tolylthioureas[11] were prepared by the action of alcoholic ammonia on phenyl and tolyl isothiocyanates.[12] The chlorophenyl-, nitrophenyl-, carboxyphenyl- and naphthylthioureas[13] were obtained by the action of ammonium thiocyanates on the hydrochlorides of the corresponding bases.

Preparation of 2-phenylamino-4-methyl-5-carbethoxythiazole.—A mixture containing 6.8 g. of phenylthiourea (0.045 mole), 2.6 g. of ethyl acetoacetate (0.02 mole) and 5.2 g. of iodine (0.02 mole) was heated under reflux for 8 hours on a water-bath and again from 12 to 16 hours after removal of the condenser. The period of heating influences the yield of the final product. The crude reaction product was kept in contact with ether, with occasional shaking, for 48 hours

TABLE I

2-ARYLAMINO-4-METHYL-5-CARBETHOXYTHIAZOLE



Compound no.	Nature of substituent, R	Thiazoles M.p., °C.	Yield, %	N, % Calcd.	N, % Found	S, % Calcd.	S, % Found	Picrate derivatives M.p., °C.	Yield, %	S, % Calcd.	S, % Found
1	Phenyl	98	67	10.68	10.42	12.21	11.96	110	89	6.52	6.29
2	*o*-Tolyl	156	54	10.14	9.86	11.59	11.48	121	85	6.34	6.28
3	*p*-Tolyl	82–84	52	10.14	10.01	11.59	11.51	117	89	6.34	6.32
4	*o*-Chlorophenyl	105	42	9.42	9.26	10.77	10.62	120	80	6.10	5.92
5	*m*-Chlorophenyl	142	45	9.42	9.18	10.77	10.55	123	85	6.10	5.89
6	*p*-Chlorophenyl	130	43	9.42	9.20	10.77	10.60	118	82	6.10	5.94
7	*o*-Carboxyphenyl	Above 250	57	9.15	8.98	10.45	10.31	115	84	6.0	5.84
8	*m*-Carboxyphenyl	Above 250	52	9.15	8.96	10.45	10.27	124	82	6.0	5.86
9	*p*-Carboxyphenyl	216–218	50	9.15	8.92	10.45	10.42	119	80	6.0	5.90
10	*m*-Nitrophenyl	158	60	13.67	13.24	10.42	10.25	115	80	5.99	5.86
11	*p*-Nitrophenyl	106	58	13.67	13.34	10.42	10.32	115	75	5.99	5.94
12	α-Naphthyl	122	60	8.97	8.84	10.25	10.08	116	82	5.93	5.82
13	β-Naphthyl	110–112	62	8.97	8.79	10.25	10.15	192	80	5.93	5.91

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(12) F. B. Dains, R. Q. Brewster and C. P. Olander, *Org. Synthesis*, **1**, 447 (1921).
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(to remove unchanged ketone which otherwise led to a gummy product). Final removal of iodine was effected by treatment with thiosulfate solution. The resulting product which was now nearly colorless was boiled with water and filtered hot. The residue was treated with concentrated ammonia to liberate the base and then filtered. The product was crystallized from 50% alcohol.

In some cases, the residue after boiling with water was gummy. In such cases, longer contacts (for 7 to 8 hours) with concentrated ammonia or even refluxing with concentrated ammonia on a water-bath was necessary. The gummy products hardened up after such treatment with only two or three exceptions. Thiazole bases which still resisted crystallization were obtained crystalline by treating their picrates with lithium hydroxide and extracting with ether.

Thiazoles derived from carboxyphenylthioureas dissolved in ammonium hydroxide. The bases, in such cases, were liberated by treatment with acetic acid.

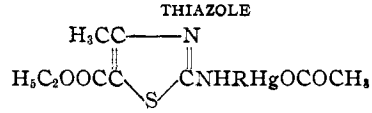
Mercuration of Thiazoles.—The thiazole (1 mole) in alcohol–dilute acetic acid solution was treated with an aqueous solution of mercuric acetate (1.3 moles) acidified with acetic acid. There was precipitation after some time. The reaction mixture was kept overnight. The precipitate was filtered and purified by repeated washing with hot water, alcohol and very dilute acetic acid.

The properties and analytical data of the resulting thiazoles are given in Table I and those of mercurated thiazoles in Table II.

The Rideal-Walker Drop Dilution method was used for the comparative antibacterial action. The bactericidal activities change from group to group as follows: chloro > naphthyl > phenyl > tolyl > nitro > carboxy.

TABLE II

2-(ACETOXYMERCURI-ARYLAMINO)-4-METHYL-5-CARBETHOXY-THIAZOLE



Compound no.	Nature of substituent, R	M.p., °C.	Yield, %	Hg, % Calcd.	Hg, % Found
1	Phenyl	241–243	87	38.46	38.29
2	*o*-Tolyl	Above 260	80	37.31	37.12
3	*p*-Tolyl	Dec. 165	83	37.31	37.09
4	*o*-Chlorophenyl	112	78	36.03	35.92
5	*m*-Chlorophenyl	235	70	36.03	35.90
6	*p*-Chlorophenyl	188	80	36.03	35.94
7	*o*-Carboxyphenyl	Above 260	72	39.68	39.15
8	*p*-Carboxyphenyl	Above 260	75	39.68	39.03
9	*m*-Nitrophenyl	178	72	35.39	35.32
10	*p*-Nitrophenyl	165	70	35.39	35.20
11	α-Naphthyl	154–156	72	35.09	34.92
12	β-Naphthyl	172	70	35.09	34.82

The maximum activities noted were 1:4000 in case of unmercurated thiazoles and 1:140,000 in case of mercurated thiazoles. Details of these investigations will be published elsewhere.

ORISSA, INDIA

[CONTRIBUTION FROM THE CLINICAL UNIT, LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE]

Application of Ion Exchange Chromatography to the Enzymatic Resolution of Amino Acids[1]

BY CARL G. BAKER AND HERBERT A. SOBER

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Conditions are described for the isolation of the optical isomers of nine representative racemic amino acids by the application of a cation exchange column separation to the products of an asymmetric enzymatic hydrolysis procedure. This separation procedure is conveniently applicable to the resolution of relatively small amounts of initial racemic substrates equivalent to 0.3 to 1.0 g. of the amino acid enantiomorph.

The enzymatic procedure for the resolution of amino acids developed in this Laboratory[2a,b] depends upon the asymmetric enzymatic hydrolysis of the N-acyl or amide derivative of the racemic amino acid. The liberated free L-amino acid is separated from the unhydrolyzed D-derivative by the addition of ethanol; the latter derivative is subsequently converted into the D-amino acid by acid hydrolysis. However, since certain amino acids, *e.g.*, isovaline, are soluble in alcohol, and hence cannot be separated in this manner, another isolation procedure was devised. This alternative procedure, employing ion-exchange chromatography for the separation of the products of the asymmetric enzymatic hydrolysis, was designed to permit the use of the small amounts of material usually involved in the synthesis of isotopically labeled amino acids. The method also has been used for the large scale preparation of the enantiomorphs of isovaline.[3]

If cation-exchange chromatography is applied to the mixture obtained after enzymatic resolution of the N-acyl derivative, the free L-amino acid is retained by the column and the unhydrolyzed D-amino acid derivative is eluted with water. After action of the enzyme upon the amide derivative, however, a weaker cation-exchange resin is used, which permits the free amino acid to pass through the column, while the unhydrolyzed derivative is retained and subsequently removed from the column with weak acid. Cation exchange therefore affords sufficiently mild conditions to avoid hydrolysis of the N-acyl or amide derivative.

Experimental

Preparation of the Ion-exchange Resin Columns.—Dowex-50[4] and Amberlite XE-64[5] in the acid phase were used. The resin as received from the manufacturer was subjected to two cycles of washing with 5 *N* HCl, water, 1 *N* NaOH, water, and followed by a final 5 *N* HCl and water wash. Washing with water in each case was continued to completion as indicated by congo red and phenolphthalein. Resin columns (see Table I for dimensions) were prepared

(1) Presented in part before the Division of Biological Chemistry at the 120th Meeting of the American Chemical Society at Atlantic City, N. J., September, 1952.

(2) (a) S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952); (b) J. P. Greenstein, S. M. Birnbaum and M. C. Otey, *ibid.*, in press.

(3) C. G. Baker, S-C. J. Fu, S. M. Birnbaum, H. A. Sober and J. P. Greenstein, THIS JOURNAL, **74**, 4701 (1952).

(4) A strong cation-exchange resin with sulfonic acid functional groups (200–400 mesh) obtained from the Dow Chemical Company, Midland, Mich.

(5) A weak cation-exchange resin with carboxylic acid exchange groups obtained from the Resinous Products Division, Rohm and Haas, Philadelphia, Penna.