1600) to which 3 mg. of lyophilized snake venom[6]) in 1 ml. of 0.2% NaCl was added. The tubes were incubated for 2 hours at 38° at which time the addition of venom was repeated and the incubation continued for 2 more hours. 0.1 ml. of purified yeast pyrophosphatase[7] was added twice, together with the venom, to those tubes which receive it. The reaction was stopped by the addition of 0.5 ml. of 10% trichloroacetic acid, the denatured protein spun off and the inorganic orthophosphate determined on the supernatant.[8]

Separation of Reaction Products on a Dowex-1 (Chloride) Column.—Twenty μmoles of ATP was incubated with 30 mg. of lyophilized snake venom as described above. At the end of the incubation the mixture was adsorbed on a Dowex-1 (chloride) column (6 cm. × 0.7 cm.^{2}). The column was washed with a little water followed by 0.01 *N* HCl containing 0.05 *N* NaCl[9] to elute the products of the incubation. About 20 fractions of 10 ml. each were collected and their optical density at 260 mμ determined. Inorganic orthophosphate and pyrophosphate were determined on aliquots of the fractions, yeast pyrophosphatase being used for the pyrophosphate assay.

Acknowledgment.—We wish to thank Dr. J. M. Buchanan for his continued interest in this work.

(6) Obtained from Ross Allen's Reptile Institute, Silver Springs, Florida.

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DIVISION OF BIOCHEMISTRY
DEPARTMENT OF BIOLOGY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE 39, MASSACHUSETTS

Identifying Isomeric Substituted 5-Aminotetrazoles by Means of Infrared Spectroscopy

BY WILLIAM G. FINNEGAN, RONALD A. HENRY AND ALLEN L. OLSEN

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1-Methyl-5-(2,6-xylyl)-aminotetrazole was reported[1] to be the product obtained from the reaction of 2,6-dimethylacetophenone with hydrazoic acid under the conditions of the Schmidt reaction[2] and from the cyclization of 1-(2,6-xylyl)-2-methyl-3-azidoguanidine in aqueous base. This latter result seemed unusual since earlier work[3] had demonstrated conclusively that the cyclizations of less highly substituted 1-aryl-2-alkyl-3-azidoguanidines yielded the isomeric 1-aryl-5-alkylaminotetrazoles. However, the 1-alkyl-5-arylaminotetrazoles are thermodynamically more stable at the temperatures normally employed during the cyclization,[4] and the possibility existed, therefore, that the cyclization of 1-(2,6-xylyl)-2-methyl-3-azidoguanidine actually yielded the more stable isomer. In order to investigate this possibility, and to confirm or refute the structure assignment of Schwartzman and Corson, the synthesis of the methyl-(2,6-xylyl)-5-aminotetrazole was repeated and the product studied.

The problem of structure assignment cannot be resolved simply by chemical test since neither isomer has definitive chemical properties. Furthermore, the synthetic route,[3] which was employed to establish unequivocally the structure of the 1-phenyl-5-alkylaminotetrazoles and which involved the catalytic debenzylation of the corresponding 1-phenyl-5-benzylalkylaminotetrazoles, would probably fail in this case because of steric hindrance in one of the intermediate steps, namely, the hydrazinolysis of the 1-(2,6-xylyl)-3,S-dimethyl-3-benzylisothiourea. For example, steric hindrance markedly retards the rate of hydrazinolysis of 1-(2,6-xylyl)-S-methylisothiourea[5] and 1-(2,6-xylyl)-3,S-dimethylisothiourea, and almost completely prevents the hydrazinolysis of 1-(2,6-xylyl)-S-methyl-3-benzylisothiourea. Another test, which was developed previously and used to differentiate between 1-phenyl-5-alkylaminotetrazoles and 1-alkyl-5-phenylaminotetrazoles, was based on the almost quantitative, thermal rearrangement of the former isomers into the latter under non-equilibrium conditions. Although the methyl-(2,6-xylyl)-5-aminotetrazole formed in the cyclization of the substituted azidoguanidine does isomerize upon heating to 180°, the result was not considered to be sufficient evidence in favor of the 1-(2,6-xylyl)-5-methylaminotetrazole structure for the following reason: Since 1-methyl-5-benzylaminotetrazole will isomerize to an equilibrium mixture and since the 2,6-xylyl group is roughly comparable to the benzyl group in electronegativity,[6] the probability of the reverse isomerization occurring (1-methyl-5-(2,6-xylyl)-aminotetrazole to 1-(2,6-xylyl)-5-methylaminotetrazole) cannot be overlooked.

Lacking an unambiguous synthesis for one of the isomers and a chemical means of identification, a proof of structure based on infrared spectra was developed. Since the aryl ring is directly conjugated with the tetrazole ring in 1-aryl-5-alkylaminotetrazoles and is not so conjugated in 1-alkyl-5-arylaminotetrazoles, the infrared spectra of a pair of these isomers should show differences related to the presence or absence of this conjugation. An examination of the spectra of 1-ethyl-5-phenylaminotetrazole and of 1-phenyl-5-ethylaminotetrazole,[3] shows distinct differences in the regions attributed to phenyl[7] and tetrazole absorptions.[8] A comparison of these two spectra with the spectra of 1-methyl-5-phenylaminotetrazole, 1-phenyl-5-methylaminotetrazole, 1-cyclohexyl-5-phenylaminotetrazole and 1-phenyl-5-cyclohexylaminotetrazole reveals that the spectra of the 1-alkyl-5-phenylaminotetrazoles have characteristic patterns in the phenyl and tetrazole absorption regions and that these patterns are different from those of the isomeric 1-phenyl-5-alkylaminotetrazoles. All of the spectral patterns for the latter isomers are similar in the phenyl and tetrazole absorption regions. Finally, the spectra of two isomeric methyl-(2-tolyl)-5-aminotetrazoles

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(2) One other example of this anomalous reaction has been described by K. F. Schmidt [U. S. Patent 1,599,493 (1926); *C. A.*, **20**, 3460 (1926)] and discussed in greater detail by P. A. S. Smith, THIS JOURNAL, **76**, 436 (1954).

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(4) R. A. Henry, W. G. Finnegan and Eugene Lieber, THIS JOURNAL, **77**, 2264 (1955).

(5) R. A. Henry, W. G. Finnegan and Eugene Lieber, *ibid.*, **76**, 88 (1954).

(6) This statement is based on the fact that the equilibrium constants for the isomerization of 1-(2,6-xylyl)-5-aminotetrazole and of 1-benzyl-5-aminotetrazole are of the same order of magnitude (ref. 4).

(7) H. M. Randall, R. G. Fowler, Nelson Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949, p. 221.

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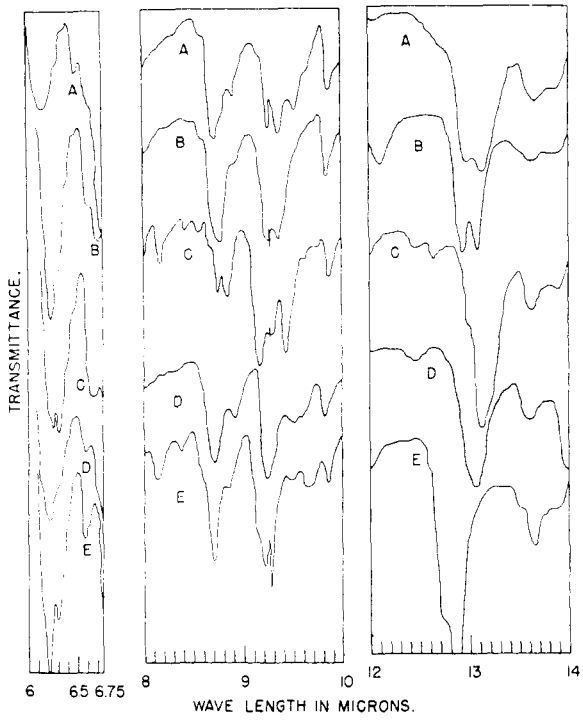

Fig. 1.—Infrared spectra: A, 1-phenyl-5-methylaminotetrazole; B, 1-phenyl-5-ethylaminotetrazole; C, 1-phenyl-5-cyclohexylaminotetrazole; D, 1-(2-tolyl)-5-methylaminotetrazole; E, 1-(2,6-xylyl)-5-methylaminotetrazole.

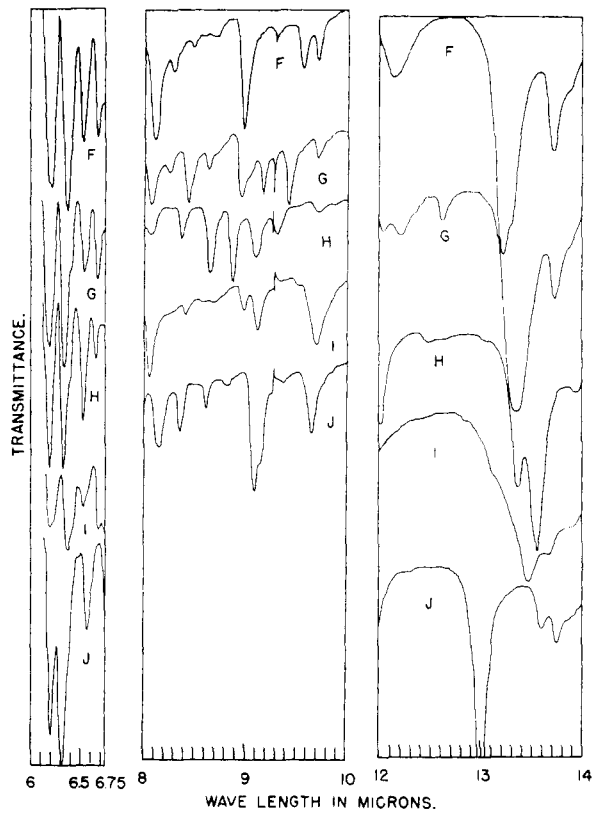

Fig. 2.—Infrared spectra: F, 1-methyl-5-phenylaminotetrazole; G, 1-ethyl-5-phenylaminotetrazole; H, 1-cyclohexyl-5-phenylaminotetrazole; I, 1-methyl-5-(2-tolyl)-aminotetrazole; J, 1-methyl-5-(2,6-xylyl)-aminotetrazole.

and of the isomeric methyl-(2,6-xylyl)-5-aminotetrazoles have been compared with the spectra for the preceding groups of compounds (Figs. 1 and 2).

TABLE I

Compound	Empirical formula	M.p., °C.	Recrys. from	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
1-(2,6-Xylyl)-3-benzylthiourea[a]	$C_{16}H_{18}N_2S$	110–111	Ethanol	71.07	71.23	6.71	6.65	10.36	10.7	11.86	12.50
1-(2,6-Xylyl)-S-methyl-3-benzyl-isothiourea	$C_{17}H_{20}N_2S$	55–56	75% EtOH	71.78	71.63	7.09	6.45	9.85	10.0	11.27	11.0
1-(2-Tolyl)-3,S-dimethylisothiourea hydroiodide monohydrate	$C_{10}H_{15}IN_2S\cdot H_2O$[b]	174–176[c]	Water	35.30	34.94	5.04	4.83			9.43	9.32
1-(2,6-Xylyl)-3,S-dimethylisothiourea nitrate	$C_{11}H_{17}N_3O_3S$	151–152	Water	48.69	48.79	6.32	6.04	15.49	15.4	11.82	11.8
1-(2,6-Xylyl)-S-methyl-3-benzyl-isothiourea nitrate	$C_{17}H_{21}N_3O_3S$	153.5–154	Water	58.76	58.88	6.09	5.72	12.10	12.0	9.23	9.50
1-(2-Tolyl)-2-methyl-3-amino-guanidine hydroiodide	$C_9H_{15}IN_4$	87–88	Nitromethane–benzene	35.30	35.66	4.94	5.33	18.30	18.6		
1-(2,6-Xylyl)-2-methyl-3-amino-guanidine hydroiodide	$C_{10}H_{17}IN_4$	183–184		37.51	36.43	5.35	5.49	17.50	17.2		
1-(2-Tolyl)-5-methylamino-tetrazole	$C_9H_{11}N_5$	153–155	Benzene	57.12	57.29	5.86	5.83	37.02	37.01		
1-Methyl-5-(2-tolyl)-amino-tetrazole	$C_9H_{11}N_5$	141–142	Water	57.12	57.25	5.86	5.58	37.02			
1-Methyl-5-(2,6-xylyl)-amino-tetrazole	$C_{10}H_{13}N_5$	110–112	Benzene	59.08	58.99	6.45	6.36	34.46	34.6		

[a] A small amount of a isomeric thiourea, m.p. 212–213°, was isolated from the reaction. *Anal.* Calcd. for $C_{16}H_{18}N_2S$: C, 71.07; H, 6.71; N, 10.36; S, 11.86. Found: C, 71.52; H, 6.99; N, 10.3; S, 11.9. [b] Calcd. for $C_{10}H_{15}IN_2S\cdot H_2O$: H_2O, 5.30. Found: H_2O, 5.40. [c] Melting point for anhydrous compound.

Although the absorption peaks of the *ortho*-substituted phenyl groups are no longer completely comparable, the tetrazole ring absorption patterns are sufficiently unchanged so as to permit structure assignments to be made for each of the isomers.

On the basis of these comparisons, the major products isolated from the cyclizations of the 1-(*o*-substituted aryl)-2-alkyl-3-azidoguanidines are the 1-aryl-5-alkyl aminotetrazoles; isomerizations of the latter at 180 to 200° yield the isomeric 1-alkyl-5-arylaminotetrazoles. Both of these conclusions are in complete agreement with observations reported previously from this Laboratory and are in disagreement with the structure assignment of Schwartzman and Corson.

Experimental[9]

The tetrazoles and their intermediates were synthesized

(9) The melting points were determined in capillary tubes and are corrected. The analyses were made by the Truesdail Laboratories, Inc., Los Angeles, Calif.

by previously published procedures.[1,3] Physical constants and analytical data of new compounds are reported in Table I. The infrared spectra were determined in Nujol mulls using a Perkin–Elmer, model 21, spectrophotometer with sodium chloride optics.

ORGANIC CHEMISTRY BRANCH
CHEMISTRY DIVISION
U. S. NAVAL ORDNANCE TEST STATION
CHINA LAKE, CALIFORNIA

The Preparation of 3-Amino-2-acetylaminofluorene and 2-Amino-7-benzoylaminofluorene[1]

BY HELMUT R. GUTMANN AND STUART W. FENTON

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For studies of the metabolism of the carcinogen 2-aminofluorene and related derivatives in progress in this Laboratory 3-amino-2-acetylaminofluorene and 2-amino-7-benzoylaminofluorene were desired. While there is no record for the synthesis of 2-amino-7-benzoylaminofluorene in the literature, the synthesis of 3-amino-2-acetylaminofluorene has been reported by Hayashi and Nakayama[2] by reduction of 3-nitro-2-acetylaminofluorene with stannous chloride to yield a product which melted at 194.5–195.5°. No data on the composition of their product were given. Attempts to prepare 3-amino-2-acetylaminofluorene by chemical reduction, either by the method of Hayashi and Nakayama[2] or with iron powder and glacial acetic acid, resulted in products which melted indefinitely starting at 130–140° and which could not be purified satisfactorily. Pure 3-amino-2-acetylaminofluorene, m.p. 225–227°,[3] was obtained by catalytic hydrogenation of 3-nitro-2-acetylaminofluorene.[2] The identity of the compound was established by elemental analysis and infrared spectrum. Treatment of the amine in cold, dilute sulfuric acid with sodium nitrite gave 1-N-acetyl-9H-fluoreno[3,2]-triazole. Formation of a triazole under similar conditions has been reported in the benzene series with *o*-aminoacetanilide.[4]

2-Amino-7-benzoylaminofluorene was obtained by reduction, with zinc dust and ethanol, of 2 nitro-7-benzoylaminofluorene. The latter compound was prepared by treating 2-amino-7-nitrofluorene[5,6] with benzoyl chloride. Diazotization of the amine followed by hydrolysis in dilute sulfuric acid gave only small amounts of 2-hydroxy-7-benzoylaminofluorene. This derivative was prepared in good yield and purity by benzoylation of the hydrochloride of 2-hydroxy-7-aminofluorene.[7]

Experimental

3-Amino-2-acetylaminofluorene.—2.70 g. of 3-nitro-2-acetylaminofluorene (0.01 mole), m.p. 201–202°,[2] N 10.4% (theory 10.4%), and 400 mg. of platinum oxide were suspended in 70 ml. of glacial acetic acid and hydrogenated[8] at 27° and 2.7 atmospheres. Hydrogen uptake was complete after 10 minutes, the observed pressure drop (2.6 lb./in.²) being close to the expected pressure drop (2.8 lb./in.²). The reaction mixture was filtered and the catalyst washed with glacial acetic acid. The filtrate was cooled in an ice-bath and rendered alkaline. The gray precipitate was collected, washed free of alkali with distilled water and dried in air. There was obtained 1.2 g. of material, m.p. 204–205°. Recrystallization of the product from 95% ethanol (75 ml./g.) gave 0.73 g. of 3-amino-2-acetylaminofluorene, m.p. 225–227° dec. Recrystallization of the product from benzene yielded long, colorless needles, m.p. 225–227° dec. The compound was soluble in dilute sulfuric and hydrochloric acid and in glacial acetic acid, but only very slightly soluble in diethyl ether. Hayashi and Nakayama[2] state that their product was readily soluble in diethyl ether.

Anal. Calcd. for $C_{15}H_{14}N_2O$: C, 75.6; H, 5.92; N, 11.8. Found: C, 75.6; H, 6.17; N, 11.8.

The infrared spectrum was determined using a Perkin-Elmer model 21 spectrometer fitted with a sodium chloride prism. The sample was prepared as a potassium bromide pellet. The following prominent absorption maxima were observed in the region 4000–1300 cm.$^{-1}$: 3400, 3320, 3260, 3020, 2920, 2840, 1650, 1620, 1585, 1530, 1490, 1470, 1455, 1435, 1405, 1370, 1310 cm.$^{-1}$.

1-N-Acetyl-9H-fluoreno[3,2]triazole.—0.78 g. of 3-amino-2-acetylaminofluorene (0.0033 mole) was dissolved in 80 ml. of 1.6 *M* sulfuric acid with slight warming on the steam-bath. The solution was filtered and the filter rinsed with 40 ml. of 1.6 *M* sulfuric acid. The solution was cooled to 10–15° in an ice-bath and 0.24 g. of sodium nitrite (0.0034 mole) in 10 ml. of distilled water was added dropwise to the rapidly stirred solution over a period of 0.5 hour. After addition of a few drops of the sodium nitrite solution the reaction mixture turned a purplish-red color and a white material precipitated. After addition of the sodium nitrite solution had been completed the suspension was stirred an additional 15 minutes. Excess nitrous acid was destroyed by addition of solid urea and the suspension stirred at room temperature 30 minutes longer. The precipitate was collected and washed free of acid with distilled water. After drying at reduced pressure over calcium chloride it weighed 0.47 g., m.p. 210–214°. The compound was recrystallized from ethanol to give long needles, m.p. 217–218°. Recrystallization from benzyl alcohol gave a product melting at 219–220°. Further recrystallization from glacial acetic acid did not change the melting point. The compound was readily soluble in ether or benzene.

Anal. Calcd. for $C_{15}H_{11}N_3O$: C, 72.3; H, 4.45; N, 16.9. Found: C, 72.0; H, 4.95; N, 16.7 (Dumas).

Nitrogen determinations by the micro Kjeldahl procedure[9] using digestion times of 2 or 8 hours gave a value of 5.76% (4 determinations).

2-Nitro-7-benzoylaminofluorene.—4.3 g. of 2-amino-7-nitrofluorene[5] (0.013 mole), m.p. 229–232°, was dissolved in 40 ml. of hot pyridine, diluted with 250 ml. of hot benzene and treated dropwise with 2.2 ml. (0.019 mole) of benzoyl chloride in 20 ml. of benzene. The solution, heated and stirred vigorously during the addition, turned deep-orange and a yellow precipitate began to form. After the addition, heating and stirring were continued for 4 hours. The mixture was allowed to stand overnight at 4°, and the precipitate was collected, washed with 10 ml. of cold benzene, then twice with cold 95% ethanol. There was obtained 5.7 g. of a yellow, fluffy material, m.p. 283–285° dec., 90% yield. Recrystallization of 0.2 g. of the compound from glacial acetic acid gave a product melting from 282–286° dec.

Anal. Calcd. for $C_{20}H_{14}N_2O_3$: C, 72.7; H, 4.27; N, 8.49. Found: C, 72.7; H, 4.29; N, 8.50.

2-Amino-7-benzoylaminofluorene.—4.0 g. (0.012 mole) of 2-nitro-7-benzoylaminofluorene, m.p. 282–286°, was ground in a mortar with 15 g. of zinc dust, and then heated under reflux with stirring with 220 ml. of 95% ethanol, 1.0 g. of calcium chloride in 10 ml. of distilled water and a trace of $CuSO_4 \cdot 5H_2O$. After 2 hours 5.0 g. of zinc dust and 50 ml. of 95% ethanol were added and heating and stirring

(1) Supported by a grant from the American Cancer Society on recommendation of the Committee on Growth, National Research Council.

(2) M. Hayashi and A. Nakayama, *J. Soc. Chem. Ind. Japan* [*Suppl.*], **36**, 127B (1933).

(3) All melting points are uncorrected.

(4) F. Bell and J. Kenyon, *J. Chem. Soc.*, 954 (1926).

(5) O. Diels, *Ber.*, **35**, 3285 (1902).

(6) F. E. Cislak and C. S. Hamilton, THIS JOURNAL, **53**, 746–749 (1931).

(7) Prepared according to a personal communication from Dr. John H. Weisburger, National Cancer Institute, National Institutes of Health.

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