pletely valid criterion of purity where the composition of the reaction mixture is similar to that of the product).

For analysis of LBF samples, the bound pantothenic acid assay has proved most satisfactory, since greater precision has been obtainable with this method. Direct analysis of LBF with *L. helveticus*, although giving variable results in our hands, has been used to confirm the identity of the compound since non-LBF materials containing pantothenic acid have occasionally been observed as contaminants in synthetic preparations.[15]

In an autoclaved pantothenic acid free medium,[11] β-aletheine possessed less than 0.5% of the activity of an equivalent amount of β-alanine in supporting growth of yeast. In a filter-sterilized medium, the corresponding activity was less than 0.07%.

β-Aletheine was not inhibitory to the growth of yeast when β-alanine was used as the growth factor. The non-utilization of β-aletheine by yeast is probably due to the impermeability of the cells to this compound. However, an alternative possibility in which pantothenic acid is first formed, and then condensed with the other moieties to give coenzyme A, is not excluded. Evidently, β-aletheine is not identical with the active principle of the incubation mixture of β-alanine with glutamic acid in the presence of resting yeast cells as reported previously.[17]

(17) T. E. King, I. G. Fels and V. H. Cheldelin, *Science*, **71**, 131 (1949).

CORVALLIS, OREGON

[FROM THE CHEMISTRY LABORATORY OF THE STATE UNIVERSITY OF IOWA]

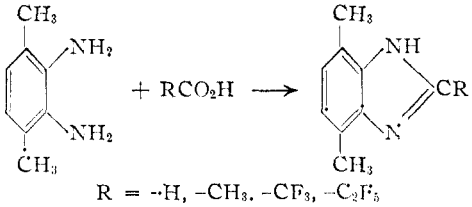
Some Substituted Benzimidazoles

BY WALTER T. SMITH, JR., AND E. C. STEINLE, JR.

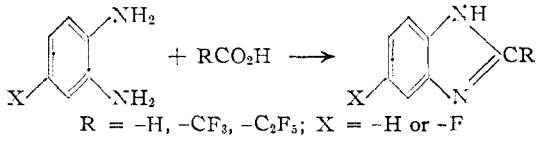
RECEIVED OCTOBER 16, 1952

4,7-Dimethylbenzimidazole, an isomer of the dimethylbenzimidazole isolated from vitamin B_{12}, has been prepared by two methods. In addition, several benzimidazoles of interest have been prepared from the diamines, *o*-phenylenediamine, 3,6-dimethyl-*o*-phenylenediamine and 4-fluoro-*o*-phenylenediamine in condensation with the acids, formic, acetic, fluoroacetic, trifluoroacetic and pentafluoropropionic.

Following the discovery that 5,6-dimethylbenzimidazole was a part of the structure of vitamin B_{12}[1] and that several of the known dimethylbenzimidazoles had varied capacities as growth stimulating agents,[2] it became desirable to prepare the remaining unreported member of the group, 4,7-dimethylbenzimidazole. Since no fluorine-containing benzimidazoles have been reported, it was also desirable to synthesize several representative compounds of this nature. Accordingly, several compounds as represented below have been prepared.



R = -H, $-CH_3$, $-CF_3$, $-C_2F_5$



R = -H, $-CF_3$, $-C_2F_5$; X = -H or -F

Difficulty was experienced in securing a sufficient supply of pure 6-nitro-2,5-xylidine, precursor of the necessary 3,6-dimethyl-*o*-phenylenediamine. It has been reported that 6-nitro-2,5-xylidene melting at 36° can be prepared in 34% yield by the nitration of N-acetyl-2,5-xylidene with mixed acids, followed by saponification of the acetyl group and steam distillation to separate the desired compound.[3] In this investigation, the 34% yield was duplicated, but the product was low-melting and resisted efforts to purify it further and was unsatisfactory for the preparation of stable 3,6-dimethyl-*o*-phenylenediamine. Neither the use of acetyl nitrate or methyl nitrate as nitration agents, nor the substitution of N-formyl-2,5-xylidine as starting material was successful in improving the yield or purity of the product. The following procedure was found to result in a superior product: sulfonation of 2,5-xylidine to give 4-amino-2,5-xylenesulfonic acid, acetylation to 4-acetylamino-2,5-xylenesulfonic acid and nitration of this compound with mixed acids, followed by hydrolysis and steam distillation. By exhaustive steam distillation, a yield of 25% of good quality 6-nitro-2,5-xylidine was isolated and a total yield of 50% was accounted for.

Reduction of 6-nitro-2,5-xylidine with zinc and sodium hydroxide or with sodium hydrosulfite gave the 3,6-dimethyl-*o*-phenylenediamine in sufficient purity (m.p. 72.5–74°) to be reasonably stable, but the yield was fairly low. Consequently, this diamine was usually isolated as the hydrochloride from benzene in 50–60% yield, and used in this form in the preparation of 2-substituted-4,7-dimethylbenzimidazoles.

The dinitration of *p*-xylene is reported to give a mixture of products containing 60–80% of the desired 2,3-dinitro-*p*-xylene.[4] This latter compound would be suitable starting material for the preparation of 4,7-dimethylbenzimidazole, but its separation from 2,6-dinitro-*p*-xylene is impractical. It seemed possible that the separation of the iso-

(1) N. G. Brink and K. Folkers, THIS JOURNAL, **71**, 2951 (1949).

(2) G. Emerson, N. G. Brink, F. W. Holly, E. Koniuszy, D. Heyl and K. Folkers, *ibid.*, **72**, 3084 (1950).

(3) M. H. Wahl, *Ann. chim.*, [11] **5**, 26 (1936).

(4) K. A. Kobe and T. B. Hudson, *Ind. Eng. Chem.*, **42**, 356 (1950).

TABLE I

Benzimidazole	M.p.,[a] °C., cor.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Diamine, mole	Reactants Acid, mole	Reactants Other, ml.	Reaction time, hr.	Yield, %
4,7-Dimethyl-[b]	266–267	$C_9H_{10}N_2$	73.94	73.33	6.90	7.08	0.0046[c]	Excess	H_2O, 4	1.5	60
2,4,7-Trimethyl	245–245.5	$C_{10}H_{12}N_2$	74.96	74.43	7.55	7.66	.0029[c]	Excess	2 *N* HCl, 10	2	76[d]
4,7-Dimethyl-2-(trifluoromethyl)-	218.5–219	$C_{10}H_9N_2F_3$	56.07	55.87	4.23	4.22	.0057[c]	0.0196	H_2O, 10	4	32.6[d]
4,7-Dimethyl-2-(pentafluoroethyl)-	211–212	$C_{11}H_9N_2F_5$	50.00	49.17	3.43	3.43	.0057[c]	0.019	H_2O, 10	4	13[d]
5(or 6)-Fluoro-	137–137.5	$C_7H_5N_2F$	61.76	61.97	3.70	3.81	.045[e]	Excess	.....	4	11.4[d,f]
5(or 6)-Fluoro-2-(trifluoromethyl)-	219–220	$C_8H_4N_2F_4$	47.07	47.26	1.97	2.05	.0198	0.021	4 *N* HCl, 25	0.75	32
5(or 6)-Fluoro-2-(pentafluoroethyl)-	192–193	$C_9H_4N_2F_6$	42.53	42.09	1.59	1.81	.0198	.021	.....	Overnight	15.9
2-(Fluoromethyl)-	132.5–134.5	$C_8H_7N_2F$	63.99	63.45	4.70	4.78	.0463	.05[g]	4 *N* HCl, 50	3	50
2-(Trifluoromethyl)-	210–210.5	$C_8H_5N_2F_3$	51.62	51.97	2.71	2.64	.0925	.10	.....	6	75.5
2-(Pentafluoroethyl)-	214–214.5	$C_9H_5N_2F_5$	45.77	46.07	2.13	2.14	.0925	.10	H_2O, 5	6	11.9

[a] Placed on Fisher–Johns m.p. block about 5° below m.p. [b] Calcd.: N, 19.17. Found: N, 19.28. [c] The dihydrochloride salt. [d] Crude. [e] Moles of 2-nitro-4-fluoroaniline used for preparation of diamine. [f] Based on 2-nitro-4-fluoroaniline. [g] Sodium salt of 90% purity.

meric dinitro-*p*-xylenes might be by-passed, since, of the two diamines obtained by reduction only the 2,3-diamino-*p*-xylene could give a benzimidazole. Such a plan depends on the easy separation of the resulting benzimidazole from either unreacted 2,6-diaminobenzimidazole or its diformyl derivative. The mixed dinitro-*p*-xylenes were reduced to mixed diamine sulfates. When the mixture of diamine sulfates was refluxed with formic acid the desired 4,7-dimethylbenzimidazole could be easily isolated in about 33% yield. This yield is based on the mixture of diamino-*p*-xylenes and is perhaps nearly twice as great if based on the estimated 60–80% 2,3-diamino-*p*-xylene present in the mixture. This method compares favorably with the lengthy procedures described above involving the preparation of pure 2,3-diamino-*p*-xylene.

In order to introduce a fluorine into the benzene ring of benzimidazole, 4-fluoro-*o*-phenylenediamine has been used as the intermediate. By the nitration of *p*-fluoroacetanilide with methyl nitrate and hydrolysis of the acetyl group, 2-nitro-4-fluoroaniline was produced in good yield. This was reduced with sodium hydrosulfite or with zinc and sodium hydroxide to give 4-fluoro-*o*-phenylenediamine in yields of 62–72% in varying degrees of purity. The highest melting point obtained was 92°. Since the compound exhibited a tendency to decompose it was characterized only as 5(or 6)-fluorobenzimidazole and was used without further purification for the preparation of other benzimidazoles.

The benzimidazoles were prepared by the condensation of the appropriate diamine or its salt with a suitable organic acid, with or without dilute hydrochloric acid as catalyst. Some details of the preparations and the yields are given in Table I.

The benzimidazoles prepared were in general stable, easily sublimed compounds. The presence of a trifluoromethyl or pentafluoroethyl group in the 2-position seemed to enhance the acidity of the 1-hydrogen, since the compounds containing these groups were soluble in dilute alkaline solutions and insoluble in dilute acid. The 2-(fluoromethyl)-benzimidazole, however, was unstable to heat, could not be sublimed and appeared to resemble an allylic halide type of compound in its sensitivity to hydrolysis.

The benzimidazoles reported here will be tested for growth-promoting activity and the results will be reported elsewhere.

The authors are grateful to the Minnesota Mining and Manufacturing Company for the samples of fluorinated acids used in this work.

Experimental

6-Nitro-2,5-xylidine.—The sulfonation of 2,5-xylidine was carried out according to the procedure for the sulfonation of *o*-toluidine[5]; the sulfonic acid was isolated in 87% yield by acidification of the solution of its barium salt.

The resulting sulfonic acid (47 g., 0.233 mole) was refluxed overnight with 250 ml. of glacial acetic acid, 40 g. of fused sodium acetate and 100 ml. of acetic anhydride to give a nearly quantitative yield of the N-acetyl derivative. This was dissolved in 250 ml. of concentrated sulfuric acid. To the resulting stirred solution was added dropwise 23 g. (16.2 ml., 0.259 mole) of concentrated nitric acid in 65 ml. of concentrated sulfuric acid. The temperature was maintained at 30° during the addition, which required 30 minutes. Stirring was continued for one hour, then the reaction mixture was poured into 275 ml. of water and the resulting mixture was refluxed for one hour to remove the acetyl and sulfonic acid groups. The mixture was diluted with water to about 1 l. and steam distilled. By chilling the distillate as collected and removing the orange crystals by filtration, two batches of product were isolated. The first, 13% yield, was impure, containing a high-melting substance. The second, 25% yield, was of satisfactory purity, melting at 33–36°. From the amount of product isolated by benzene extraction of a portion of the steam distillate after filtration, it is estimated that the total filtrate contained an additional 12% yield. Thus the total yield accounted for was 50%.

3,6-Dimethyl-*o*-phenylenediamine.—To 2.8 g. (0.035 mole) of 6-nitro-2,5-xylidine suspended in 60 ml. of boiling alcohol was added in portions 28 g. (0.133 mole) of sodium hydrosulfite suspended in 50 ml. of water, with stirring. After the addition was complete, the mixture was heated and stirred for 30 minutes. The solution was filtered and cooled, and extracted with six 50-ml. portions of benzene. When gaseous hydrogen chloride was bubbled into the dried extracts, 4.4 g. (60%) of the hydrochloride of 3,6-dimethyl-*o*-phenylenediamine was obtained. Alternately, the free base, m.p. 72.5–74°, could be isolated in 31% yield by concentration of the benzene extracts followed by addition of Skellysolve H. This reduction was also carried out with zinc and sodium hydroxide.

4-Fluoro-*o*-phenylenediamine.—*p*-Fluoroacetanilide was prepared by the reduction of *p*-fluoronitrobenzene with iron and hydrochloric acid, steam distillation of the reaction mixture, and acetylation of the *p*-fluoroaniline in the steam distillate. The over-all yield was nearly quantitative. The *p*-fluoroacetanilide was nitrated by the method of Wilkinson

(5) C. F. H. Allen and J. A. Van Allan, *Org. Syntheses*, **27**, 88 (1947).

and Finar,[6] using methyl nitrate[7] rather than ethyl nitrate as the nitrating agent. After hydrolysis[6] of the 2-nitro-4-fluoroacetanilide with 8 *N* hydrochloric acid, the reaction mixture was steam distilled. From the distillate, 2-nitro-4-fluoroaniline melting at 93° was isolated by filtration in 81.3% yield.

To a solution of 10 g. (0.064 mole) of 2-nitro-4-fluoroaniline in 20 ml. of ethyl alcohol and 15 ml. of 30% sodium hydroxide solution was added in portions and with shaking 15 g. (0.23 gram atom) of zinc dust. The mixture was heated on the steam-bath for 30 minutes, filtered and extracted with two 100-ml. and five 50-ml. portions of benzene. The extracts were concentrated to about 50 ml., diluted with an equal volume of Skellysolve H, cooled and filtered to give 5 g. of 4-fluorophenylenediamine, m.p. 89–91°. An additional 0.8 g. was isolated by concentration of the mother liquor, bringing the total yield to 72%. The product was not further purified but was used immediately in condensations to produce benzimidazoles.

By reduction of 2-nitro-4-fluoroaniline with sodium hydrosulfite a product melting at 92° was obtained in 37% yield.

Benzimidazoles.—The benzimidazoles were prepared by heating a mixture of the appropriate diamine or its hydrochloride with an excess of the desired acid, with or without dilute hydrochloric acid or water. If the reaction mixture was sufficiently liquid, it was refluxed; if it formed a cake, it was warmed on the steam-bath. Most of the crude products were isolated by filtration from the cooled reaction mixture after it was made just basic to litmus with 10% sodium hydroxide solution or 6 *N* ammonium hydroxide. They were purified by recrystallization from aqueous alcohol and sublimation. If the sublimate was colored or not pure, it was recrystallized.

Exceptions to the above procedure were as follows: 5(or 6)-fluorobenzimidazole was best recrystallized from benzene and Skellysolve H; 5(or 6)-fluoro-2-(trifluoromethyl)-benzimidazole was isolated by benzene extraction from the reaction mixture after it was made just slightly acid to litmus and the product was recrystallized from benzene and Skellysolve H and sublimed; the reaction mixture of 5(or 6)-fluoro-2-(pentafluoroethyl)-benzimidazole was recrystallized directly from benzene and Skellysolve H; the crude product was extracted with 6 *N* ammonium hydroxide and the material obtained by acidification of the extract was sublimed; 2-(fluoromethyl)-benzimidazole was recrystallized from benzene and Skellysolve H and could not be sublimed.

Alternate Preparation of 4,7-Dimethylbenzimidazole.—The mixture of 2,3-dinitro-*p*-xylene and 2,6-dinitro-*p*-xylene obtained by the dinitration of *p*-xylene with fuming nitric acid[8] was reduced with iron powder and hydrochloric acid according to the procedure for the reduction of dinitrotoluene,[9] the product, consisting of mixed diamino-*p*-xylenes, was isolated as the sulfate salts in 55% yield. The condensation of this product with two moles of dilute formic acid, according to the general procedure for the preparation of benzimidazoles, resulted in a 32.8% yield of 4,7-dimethylbenzimidazole, identified by melting point and mixed melting point with a sample prepared from 3,6-dimethyl-*o*-phenylenediamine.

(6) J. H. Wilkinson and I. L. Finar, *J. Chem. Soc.*, 288 (1948).

(7) A. P. Black and F. H. Babers, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 412.

(8) R. Fittig, W. Ahrens and L. Mattheides, *Ann.*, **147**, 16 (1868).

(9) S. A. Mahood and P. L. Schaffner, *Org. Syntheses*, **11**, 32 (1931).

IOWA CITY, IOWA

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY COLLEGE, CORK, IRELAND]

Studies in the Pyrazole Series. II. The 1-Nitroguanyl Type

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RECEIVED NOVEMBER 24, 1952

The reaction between acetylacetone and nitroaminoguanidine results in both hydrazone and pyrazole formation, the latter being the main process involved. The major product, *viz.*, 3,5-dimethyl-1-nitroguanylpyrazole, is the prototype of a new class of pyrazoles—the 1-nitroguanyl type. With iodine, denitroguanylation and substitution are observed; with chlorine and bromine substitution alone. When these latter halogenations are performed in alkaline media, denitroguanylation again accompanies the substitution. Nitration, depending upon the reagents used, may afford either the 4-nitro-1-nitroguanyl- or the 4-nitro-1-unsubstituted-3,5-dimethylpyrazoles. Reduction caused either disarrangement or denitroguanylation, no pyrazoline formation being observed. Finally, hydrazinolysis and ammonolysis take place, to extents depending on the basicity of the amine used and result in the symmetrical fission of the material, the nitroguanyl side-chain being incorporated in the amino and hydrazino residues.

Reaction of an acylhydrazide ($RN''HN'H_2$) with β-diketones results in (i) hydrazone formation, in which the N′-atom alone of the hydrazide molecule is concerned (primary condensation), or (ii) pyrazole formation, in which both the N′- and N″-atoms of the hydrazide are involved (secondary condensation). The relative extents to which primary and secondary condensations occur depend upon the hydrazide concerned and may be employed for characterization purposes. A condensibility coefficient or the "condensive aptitude" (C_A) of a hydrazide may then be defined as the numerical ratio between the weights (in molar proportions) of primary condensation products and secondary condensation products, formed under specified conditions.

The secondary condensation process occurs *via* $(N \rightarrow \overset{+}{C})$ chelation, followed by dehydration, and hence the extent of pyrazole formation is in reciprocal ratio to the electrophilic activity of the acyl group. The greater the electrophilic power of the acyl group, the greater should be the C_A value of the corresponding hydrazide.

In the acyl hydrazides considered in the present series, namely, where R varies from $-C(=O)-NH_2$ (A), to $-C(=S)-NH_2$ (B), to $-C(=\overset{+}{N}H_2)-NH_2$ (C), to $-C(=NH)-NHNO_2$ (D), the electrophilic activities of the groups may be arranged as follows C > A ≃ D > B. The estimation of the relative electrophilic powers of A and D from theoretical considerations alone is difficult, because the weaker $-I$ effect of the imino grouping in D is counteracted to a certain extent by the weaker $+E$ effect of its nitroamino group.

Use of the C_A concept, with its electronic implications, clarifies the uncertainty of the relative electrophilicities of A and D. The present experimental results indicate the order of electrophilicity to be C > D > A > B. (i) The C_A value for semicarbazide is definitely < 0.3 (we found its