The Reaction of Thyroxine with Aromatic Isocyanates

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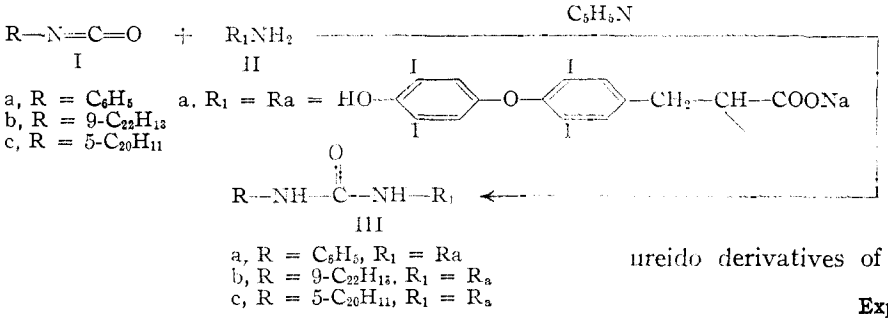
During the investigation of the influence of thyroxine on the chemical carcinogenesis as induced by 1,2,5,6-dibenzanthracene[1,2] and 3,4-benzpyrene, a stable compound containing both the thyroxine and the 1,2,5,6-dibenzanthracene molecules essentially intact was desired. In our previous studies[3-6] involving these carcinogenic hydrocarbons, the reaction of the isocyanate (I) with the amino compounds (II) to form various ureido compounds (III) was utilized.



This reaction was found to be applicable in the case of thyroxine.

The method employed was essentially that reported previously[1,2,6] in which the isocyanate (I) and the anhydrous thyroxine (IIa) were allowed to react in anhydrous pyridine at 25°. The compound, 1,2,5,6-dibenzanthranyl-9-ureidothyroxine (IIIb), formed from the 1,2,5,6-dibenzanthranyl-9-isocyanate (Ib) and the thyroxine still possessed characteristic physiological activity but no longer possessed the carcinogenic activity of the 1,2,5,6-dibenzanthracene in mice.[2,7]

This retention of physiological activity may be interpreted as evidence supporting the non-substitution of the phenolic hydroxyl group when equivalent amounts of the reactants were used. As pointed out by Ashley and Harington[8] the acetyl derivative of thyroxine which Swingle, Helff and Zwemer[9] claimed did not stimulate metabolism was in reality the ethyl ester of diacetylthyroxine. The present finding is in keeping with the evidence of Niemann, *et al.*,[10,11] that the phenolic hydroxyl group, free to be converted to a quinoid form is, essential for thyroxine activity, even, as in our case, despite the amino substitution with the relatively large group, 1,2,5,6-dibenzanthranyl-9-ureido-.

Further evidence that linkage occurs preferentially at the amino group rather than at the phenolic hydroxyl resulted from the Sorenson titration[12] of the thyroxine and the thyroxine derivatives. The sodium salt of *d,l*-thyroxine was converted to *d,l*-thyroxine by treatment with ethanolic acetic acid. Formol titration of this material gave almost the theoretical value for free amino group nitrogen. Conversion of the sodium salt of either phenylureidothyroxine (IIIa) or 1,2,5,6-dibenzanthranyl-9-ureidothyroxine (IIIb) to the corresponding acid with ethanolic acetic acid gave a compound with almost zero free amino group nitrogen.

The lack of carcinogenic activity of the 1,2,5,6-dibenzanthranyl-9-ureidothyroxine (IIIb) is in agreement with the results we have found with other ureido derivatives of carcinogens.[13,14]

Experimental

Sodium Salt of *d,l*-Thyroxine.—The sodium salt of *d,l*-thyroxine (British Drug Houses) was recrystallized from absolute ethanol and dried at 30° (1 mm.) over phosphorus pentoxide for eight hours.

***d,l*-Thyroxine.**—To 200 mg. of the sodium salt of thyroxine in 125 ml. of absolute ethanol was added 40 ml. of glacial acetic acid and the resulting solution allowed to stand at 25° for 16 hours. Evaporation of the reaction mixture to 60 ml. gave 113 mg. of colorless leaf-like crystals, m.p. 227–229° (dec.). This material gave the theoretical value for free amino group nitrogen on formol titration.

Sodium Salt of Phenylureidothyroxine (IIIa).—To a suspension of 0.09 g. (0.11 millimole) of the sodium salt of thyroxine in 2.0 ml. of anhydrous pyridine was added a solution of 0.016 g. (0.12 millimole) of phenyl isocyanate in 2.0 ml. of anhydrous pyridine and the resulting suspension agitated at 0–10° for two hours during which time the thyroxine dissolved. Removal of the solvent and excess isocyanate under reduced pressure gave 0.099 g. of a beige-colored amorphous solid, m.p. 202–204° (dec.). This material on recrystallization from pyridine-diethyl ether melted at 210.5–213° (dec.).

Anal. Calcd. for $C_{22}H_{15}O_5N_2I_4Na$: N, 3.05; I, 55.3. Found: N, 3.01; I, 55.1.

Phenylureidothyroxine.—Treatment of 0.25 g. of the sodium salt of phenylureidothyroxine with alcohol and acetic acid gave an almost colorless solid, m.p. 190–194° (dec.). This material gave a free amino group nitrogen value on formol titration of 0.7% of that of thyroxine. This is essentially zero within experimental error.

Sodium Salt of 1,2,5,6-Dibenzanthranyl-9-ureidothyroxine (IIIb).—To a solution of 0.26 g. (0.81 millimole) of 1,2,5,6-dibenzanthranyl-9-isocyanate (4) in 15.0 ml. of anhydrous pyridine was added 0.65 g. (0.81 millimole) of the sodium salt of thyroxine and the resulting suspension agitated at 20° for 20 hours. The reaction mixture was filtered, the residue washed with a total of 45 ml. of absolute diethyl ether to remove any uncombined isocyanate and then with a total of 40 ml. of absolute alcohol to remove any uncombined thyroxine. The product weighed 0.71 g. (77% yield) and melted at 191° (dec.).

(1) R. Bather, W. R. Franks and A. Rodgman, data presented at the Thirteenth Annual Meeting of the Canadian Physiological Society, Montreal, Canada, October 14–15, 1949.

(2) R. Bather and W. R. Franks, data presented at the Fifteenth Annual Meeting of the Canadian Physiological Society, Kingston, Ontario, October 26–27, 1951.

(3) H. J. Creech and W. R. Franks, *Am. J. Cancer*, **30**, 555 (1937); **35**, 203 (1939).

(4) H. J. Creech and W. R. Franks, THIS JOURNAL, **60**, 127 (1938).

(5) H. J. Creech and R. N. Jones, *ibid.*, **62**, 1970 (1940); **63**, 1661 (1941).

(6) W. R. Franks and A. Rodgman, data to be published.

(7) R. Bather and W. R. Franks, data to be published.

(8) J. H. Ashley and C. R. Harington, *Biochem. J.*, **23**, 1178 (1929).

(9) W. W. Swingle, O. M. Helff and R. L. Zwemer, *Am. J. Physiol.*, **70**, 208 (1924).

(10) C. Niemann and C. E. Redemann, THIS JOURNAL, **63**, 1549 (1941).

(11) C. Niemann and J. F. Mead, *ibid.*, **63**, 2685 (1941).

(12) J. H. Northrop, *J. Gen. Physiol.*, **3**, 715 (1920); *ibid.*, **9**, 767 (1925).

(13) W. R. Franks, A. Rodgman and K. A. Brown, *Annual Report of the Ontario Cancer Treatment and Research Foundation*, 53 (1948).

(14) W. R. Franks, J. I. Manser and A. Rodgman, *ibid.*, 75 (1949).

Anal. Calcd. for $C_{36}H_{23}O_5N_2I_4Na$: N, 2.55; I, 45.4. Found: N, 2.51; I, 45.2.

1,2,5,6-Dibenzanthranyl-9-ureidothyroxine.—Treatment of the sodium salt of the 1,2,5,6-dibenzanthranyl-9-ureidothyroxine with ethanolic acetic acid as above gave a 73% yield of the free acid, m.p. 177–180° (dec.). Formol titration of this material indicated negligible free amino group nitrogen.

Sodium Salt of 3,4-Benzpyrenyl-5-ureidothyroxine (IIIc).—One hundred milligrams (0.34 millimole) of 3,4-benzpyrenyl-5-isocyanate[15] and 0.272 g. (0.34 millimole) of the sodium salt of thyroxine in 10 ml. of anhydrous pyridine were treated as described above. The product, weight 0.155 g., yield 42%, darkened at 160° and melted with decomposition at 171–172°.

Anal. Calcd. for $C_{38}H_{21}O_5N_2I_4Na$: N, 2.56; I, 46.4. Found: N, 2.51; I, 46.2.

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(15) H. J. Creech, THIS JOURNAL, **63**, 576 (1941).

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Replacement of the Primary Aromatic Amino Group by Hydrogen Using Diazonium Fluoborates

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Many reagents have been used to replace the primary aromatic amino group by hydrogen; an excellent review of these methods has been given by Kornblum.[1a] No one of these methods is universally applicable, and the yields vary considerably with both the method and the amine used; all of these deaminations are carried out using the diazotized amine. Usually the diazonium salt is not isolated; occasionally, however, stabilized dry diazonium salts have been used. Thus Hodgson and Marsden[2] stabilized diazotized amines with naphthalene-1,5-disulfonic acid or 2-naphthol-1-sulfonic acid and decomposed the resulting dry salt with ethanol in the presence of zinc or copper; they reported yields of the order of 90%. However, some workers have been unable to duplicate these results.[3] Leslie and Turner[4] obtained a 78% yield of 2-nitro-3′-bromobiphenyl from 4,4′-diamino-2-nitro-3′-bromobiphenyl by decomposing the bis-diazonium fluoborate salt in absolute ethanol containing sulfuric acid. In this connection, it is of interest that Niemann, Benson and Mead[5] report the incidental preparation of about 7% of ethyl 3-fluoro-4-methoxybenzoate simultaneously with the formation of ethyl 3,5-difluoro-4-methoxybenzoate in about 28% yield by the Schiemann reaction[6] from 2-methoxy-3-fluoro-5-carbethoxybenzenediazonium fluoborate, some deamination occurring along with fluorine substitution. Some light may be thrown on the reason for this deamination by the observation of Schmelkes and Rubin[7] who noted that if all the methanol used in washing the diazonium fluoborate was not completely removed, some *p*-nitrotoluene was obtained in addition to the expected 2-fluoro-4-nitrotoluene in preparing the latter by the Schiemann reaction.

With the above facts in mind, it seemed desirable to see if more consistent yields in the deamination reaction could be obtained using the diazonium fluoborates. These salts can be obtained in good yield and in excellent purity and, in addition, most of them are quite stable.[6] This paper reports the results obtained by decomposing several different types of diazonium fluoborates with ethanol in the presence of zinc; investigation of the reaction of diazonium fluoborates with hypophosphorus acid and other deaminating reagents is underway. The yields obtained (based on the diazonium fluoborate used) are reported in Table I; they are in most cases between 70–80%. The heterocyclic amines used gave quite low yields. Interestingly enough, the yield of benzene from benzenediazonium fluoborate was rather poor (46.5%); in this case, a 1% yield of biphenyl was produced—the only instance in which a coupling reaction occurred. Another curious fact about this reaction was that appreciable amounts of diethyl acetal were formed; this compound was not observed in any of the other deaminations.

TABLE I

Amine	Diazonium fluoborate Yield, %	Diazonium fluoborate Dec. point, °C.	Product obtained	Yield of product from diazonium fluoborate, %
Aniline	91	104	Benzene	46.5[a]
o-Toluidine	88	104	Toluene	82.2
m-Toluidine	84	104	Toluene	73.7
p-Toluidine	90	106	Toluene	84.8
o-Chloroaniline	91	166	Chlorobenzene	79.4
m-Chloroaniline	98	148	Chlorobenzene	82.6
p-Chloroaniline	89	153	Chlorobenzene	85.2
p-Phenetidine	80	103	Phenetole	71.0
Ethyl *p*-aminobenzoate	97	95	Ethyl benzoate	69.2
p-Nitroaniline	88	141	Nitrobenzene	80.0[b]
Benzidine	90	142	Biphenyl	82.5
β-Naphthylamine	93[c]	103	Naphthalene	64.1
m-Aminobenzotrifluoride	87	148	Benzotrifluoride	74.3
2-Bromo-4-methylaniline	87	148	*m*-Bromotoluene	69.0
6-Aminoquinoline	95	85–93	Quinoline	34.0
3-Aminoquinoline	91	93	Quinoline	31.3
3-Aminopyridine	..	15	Pyridine	26.3[d]

[a] A 1% yield of biphenyl was also obtained. [b] In this run, zinc was omitted; using zinc, the yield was only 58%. [c] Pure β-naphthylamine is necessary for preparation of stable diazonium fluoborate. [d] A 12.2% yield of 3-ethoxypyridine was also obtained.

The use of zinc seems to catalyze the reaction and also to cut down the formation of ethyl ether, confirming the observation of Hodgson[2]; only in

(1) The work reported in this paper is taken from the master's thesis submitted by Jack R. Graham to the Graduate School of the University of North Carolina, June, 1952.

(1a) N. Kornblum, in "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 262.

(2) H. Hodgson and E. Marsden, *J. Chem. Soc.*, 207 (1949).

(3) Reference 1a, page 285.

(4) M. Leslie and E. Turner, *J. Chem. Soc.*, 1590 (1933).

(5) C. Niemann, A. Benson and J. Mead, THIS JOURNAL, **63**, 2204 (1941); also a private communication from Dr. Niemann.

(6) A. Roe, in "Organic Reactions," Vol. V, John Wiley and Sons, Inc., New York, N. Y., 1949, p. 193.

(7) C. Schmelkes and M. Rubin, THIS JOURNAL, **66**, 1631 (1944).