unstable on exposure to air. *Anal.* Calcd. for $C_{15}H_{19}O_2N$: C, 73.44; H, 7.79; N, 5.71. Found : C, 73.86; H, 8.25; N, 5.34.

The pKa′ (12.0) of this base was calculated from its ultraviolet absorption spectrum (Fig. 2, 1～3).

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Summary

The enamine, 9,10-dimethoxy-3,4,6,7-tetrahydro-2*H*-benzo[*a*]quinolizine, which was isolated by basifying the aqueous solution of the corresponding immonium chloride, was proved to be reconverted into the immonium cation when dissolved in 85% ethanol to a concentration of ca. $10^{-4}M$. The equilibrium between the two is discussed based on its ultraviolet absorption spectra at various pH values.

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Yoshio Sakurai and Keiichi Itō : Paper Chromatographic Detection of Nitrogen Mustard.

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The detection of nitrogen mustard derivatives on paper chromatogram has hitherto been carried out with the Dragendorff reagent but this method was proved not to be sensitive enough for most biological work. For instance, it was difficult with this reagent to detect less than 50 γ of N-methyl-bis(2-chloroethyl)amine (HN_2).

This paper deals with an improved method, which is based on a rapid and characteristic reaction between nitrogen mustard and compounds having a mercapto group. For this purpose, an alkaline solution containing 6-mercapto-2-naphthol*2 was used which was prepared immediately before use by reduction of Seligman's reagent (6,6′-dithiodi(2-naphthol)[1]) with sodium amalgam or by alkaline hydrolysis. It was very easy to detect the spots on paper because the combined product as well as the reagent itself could be easily stained on paper by diazo-coupling reaction with diazonium salt.

In practice, ethanol solution of nitrogen mustard hydrochloride was spotted on a paper strip (Toyo Roshi No. 50, 2×40 cm.) and, after drying, just on the same place, one drop of alkaline solution of Seligman's reagent (1.0 g. in 10 cc. of 10% sodium hydroxide solution) was doubly spotted. The chromatogram was immediately run for 15～17 hours by ascending method, employing the upper layer of a ternary mixture of butanol, acetic acid, and water (80 : 20 : 100) as the solvent system. The paper strips were dried, sprayed with 0.5% *p*-diazobenzenesulfonic acid in 50% ethanol, and then exposed to ammonia gas. Four pink or orange spots appeared instantly, Rf values of which were determined respectively as 0.28 (A), 0.47 (C), 0.58 (D), and 0.90 (E).

In order to identify each spot, the chromatograms obtained under various conditions were examined by comparing the results of staining with three kinds of developing reagent, viz. the diazonium salt, Dragendorff, and sodium nitroprusside reagents, as shown

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*2 Use of free thiol as a reagent was avoided in this procedure because of its extreme instability during preservation.

1) R. J. Barret, A. M. Seligman : Science, **116**, 324(1952); T. Zincke, R. Derser : Ber., **51**, 354(1918).

in Table I.

Table I. Rf Values on Chromatogram

No.	Substance spotted*	Rf value (A)	(B)	(C)	(D)	(E)	Developing reagent*
1	S					0.90	DBS
2	S(reduced with Na-Hg)	0.28			0.59	0.89	〃
3	S(dissolved in NaOH)	0.28			0.58	0.90	〃
4	〃	0.28			0.58	0.90	N
5	S(dissolved in NaOH)+HN_2	(0.28)		0.47	0.58	0.90	DBS
6	〃		0.31	0.46			D
7	HN_2		0.32				〃

* Abbreviations used :

S : Seligman's reagent
HN_2 : Nitrogen mustard
DBS : *p*-Diazobenzenesulfonic acid (0.5%)
N : Sodium nitroprusside (5%)
D : Dragendorff reagent

These five spots (given as A, B, C, D, and E in the table) were assigned according to their reactions with each of the developing reagent, (A) to that of 6-mercapto-2-naphthol produced by alkaline hydrolysis of Seligman's reagent, (C) to reaction product of HN_2 with the thiol, (D) to probable oxidized product of the thiol, possibly 2-hydroxy-6-naphthalenesulfinic acid which might have been formed by disproportionation of Seligman's reagent in alkaline medium, (E) to Seligman's reagent itself, and (B) to the unchanged HN_2.

It could be reasonably said that the spot (C) represented the location of the 6-alkylthio-2-naphthol formed by alkylation by HN_2, because it appeared either orange with the Dragendorff reagent or pink with the diazonium salt, but remained colorless with sodium nitroprusside. In case of using a comparatively large amount of HN_2 on the paper, the spot of the thiol (A) was no longer found after development and the spot (C) appeared in its stead. On the contrary, the spot (D) did not disappear even with the excess of HN_2 used. Probably the sulfinic acid could not be esterified by HN_2 under such a condition.

It was proved that this procedure enables detection of 1.0 γ of HN_2 or HN_2 dissolved in a concentration of 0.1 mg./cc., if the spotting on the paper was repeated on the same place as many times as required.

The method is believed to be generally available for the derivatives of nitrogen mustard.

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