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Further Characterization and Isolation Studies in the Muscarine Series*

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A description is given of the separation of muscarine from choline using ion exchange chromatography on Dowex 50×8 resin. Muscarine tetraphenylboron is also described; the interpretation of the infrared spectrum of this compound is given.

The separation of muscarine from large quantities of choline was the main problem in the isolation and purification of muscarine from the fly mushroom, *A. Muscaria* L. Chromatography on cellulose[1, 2, 3] and norite[4] columns has recently been successfully used for this purpose.

In our search for a simpler preparation of muscarine we investigated a number of cation exchangers. The best results were obtained with ion exchange chromatography according to Moore and Stein[5]. This method made it possible to separate muscarine from choline in a mixture of crude chlorides of the quaternary bases[6], using 2.5 *N* hydrochloric acid as an eluent. The isolation of muscarine by using ion exchange resins has also been described by Kuehl *et al.*[7] These authors used Amberlite IRC-50 in the hydrogen form.

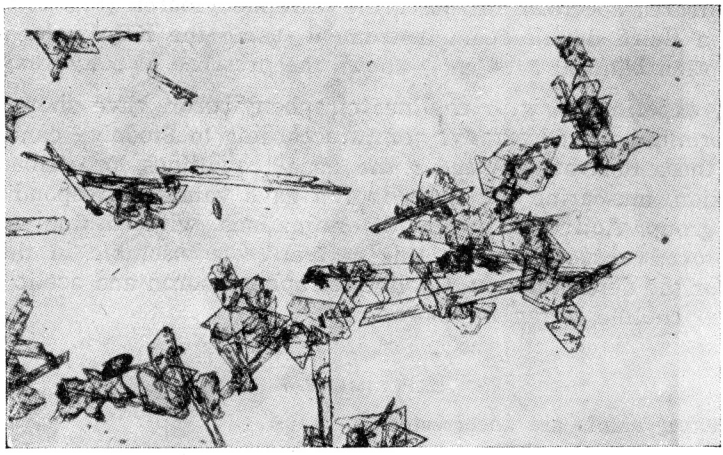

Fig. 1. Muscarine tetraphenylboron (×50)

* Communication No 63 from this Laboratory, 6th contribution in the Muscarine Series.

It his recent paper[4], Kögl *et al.* proposed an entirely new structural formula for muscarine as the quaternary trimethylammonium chloride of 2-methyl-3-hydroxy-(aminomethyl)tetrahydrofuran, and presented strong evidence in favour of it; in the same paper the authors characterized muscarine as the chloride, reineckate, chloroaurate, *p*-chlorobenzoyl- and *p*-iodobenzoyl chloroaurates, and as muscarine tetraphenylboron.

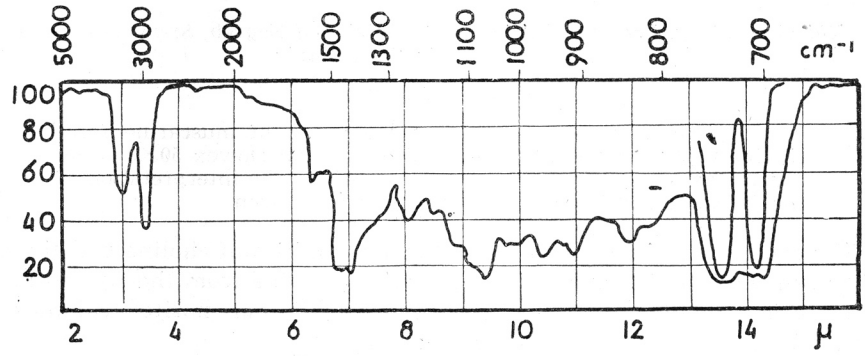

Fig. 2.

In our characterization of muscarine we used sodium tetraphenylboron[8] as a reagent. Tetraphenylboron derivatives have recently been used for the characterization of organic bases and alkaloids[10, 17]. In our case muscarine tetraphenylboron was a nonhygroscopic, well crystallized compound (Fig. 1) of remarkable stability.

The infrared spectrum of muscarine tetraphenylboron (Fig. 2) was determined on a Baird double-beam instrument, using the KBr wafer technique. The absorption band at 3424 cm.$^{-1}$ shows the presence of a hydroxyl group[11].

Model experiments with choline tetraphenylboron have shown that the micro determination of hydroxyl groups according to Stodola[12] can be carried out with these derivatives. Under the usual conditions, prescribed for this determination, muscarine tetraphenylboron gave values corresponding to one hydroxyl group. Active hydrogen determinations with lithium aluminium hydride proved unsuccessful, as this derivative is insoluble in the solvents required for the determination. Choline tetraphenylboron and acetic anhydride gave acetyl choline tetraphenylboron.

EXPERIMENTAL

All melting points are uncorrected.

Small Scale Separation of Quaternary Bases from A. Muscaria *on Dowex 50 × 8 Ion Exchange Resin*

Dowex 50 × 8 resin (200—400 mesh) was treated in turn with 8 *N* hydrochloric acid, water, 2 *N* sodium hydroxide, and 1.5 *N* hydrochloric acid. The column was

also prepared using 1.5 *N* hydrochloric acid. The ion exchange resin (250 g.) in the column (1.7 × 62 cm.) was washed with 1.5 *N* hydrochloric acid during 48 hours at a flow rate of 4 ml./hr. The column prepared in this manner was used for the separation of quaternary bases[2] (20 mg. in 20 ml. of water), at the same flow rate. The results of the separation are given in Table 1.

TABLE 1

Eluent	Total volume of eluent	Number of fractions	R_f[12]	KJ + J reaction[13]	Biological activity in M. U./per mg.[14]
1.5 *N* HCl	100 ml.	1	—	—	—
2.5 *N* HCl	290 ml.	2 — 3	—	+	—
2.5 *N* HCl	36 ml.	4 — 13	0.14	+	—
2.5 *N* HCl	52 ml.	14 — 27	—	—	—
2.5 *N* HCl	48 ml.	28 — 40	—	—	100 — 700
2.5 *N* HCl	36 ml.	41 — 50	—	—	1000 — 2500
2.5 *N* HCl	52 ml.	61 — 64	—	—	600 — 100

The fractions with muscarinic activity were combined and evaporated to dryness. The residue showed R_f values 0.24 (muscarine) and 0.18[14]. By using wider columns correspondingly larger quantities of quaternary bases could be separated.

Preparation of Muscarine Tetraphenylboron

Muscarine chloride (34.3 mg.) prepared from the chloroaurate[2] following Dudley[8], was dissolved in water (4 ml.), acidified with acetic acid (litmus), and a 0.1 *M* aqueous solution of sodium tetraphenylboron[8] added, until the filtrate remained clear on further addition of the reagent. After standing at 0° for 24 hours the precipitate was filtered through a standard porous porcelain filter crucible, washed with a few drops of dilute acetic acid and dried overnight in a high vacuum at room temperature. *Muscarine tetraphenylboron* was obtained in a yield of 60 mg. (79%), m. p. 154—155°. Several recrystallizations from acetone-water (1 : 1) gave colourless prisms (Fig. 1), yield 38 mg., with the constant m. p. 172—173°. (Infrared absorption spectrum, Fig. 2) (Lit. m. p. 174—175° [4], but without good analytical values).

Anal. 8.15 mg. subst.: 23.97 mg. CO_2, 6.06 mg. H_2O
5.70 mg. subst.: 0.15 ml. N_2 (19.2°, 757 mm.)
$C_{33}H_{40}O_2NB$ (493.48) calc'd.: C, 80.21; H, 8.17; N, 2.84%
found: C, 80.23; H, 8.32; N, 3,06%
OH-determination (Stodola[12]):
10.18 mg. subst.: 0.0224 mmoles AcOH
0.381 mg. OH
calc'd.: OH, 3.45%
found: OH, 3.74%

Choline Tetraphenylboron, Determination of Hydroxyl Groups

Choline tetraphenylboron was prepared according to Marquardt and Vogg[17].
Anal. OH-determination (Stodola[12]):
22.545 mg. subst.: 0.0571 mmoles AcOH, 0.971 mg. OH
18.778 mg. subst.: 0.0503 mmoles AcOH, 0.856 mg. OH
calc'd: OH, 4.02%
found: OH, 4.31, 4.56%

Preparation of Acetylcholine Tetraphenylboron from Choline Tetraphenylboron

Choline tetraphenylboron (100 mg.) was heated with freshly distilled acetic anhydride (4 ml.) for one hour on a water bath, and lyophilized. The pale

yellow crystalline *acetylcholine tetraphenylboron* was recrystallized from acetone-water (1 : 1) and was identical, according to m. p., mixed m. p. and elemental analysis, with the compound prepared from acetylcholine chloride and sodium tetraphenylboron[17].

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IZVOD

Izolacija i karakterizacija muskarina, daljnje studije

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Pomoću kromatografije na ionskom izmjenjivaču Dowex 50 × 8 odijeljen je muskarin od holina.

Priređen je muskarin tetrafenil bor, i interpretiran je infra-crveni spektar toga spoja.

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