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Syntheses of Chlorpromazine Undecahydrododecaborate and Nonahydrodecaborate—Promising Agents for Neutron Capture Therapy of Malignant Melanoma

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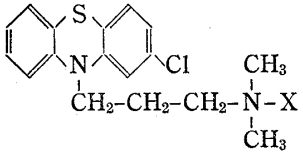
It has been suggested by dermatologists that boron compounds of chlorpromazine are promising agents for neutron capture therapy of malignant melanoma. The compounds are desired to have a high boron content, good stability, and sufficient solubility in water. To fulfil these requirements, chlorpromazine undecahydrododecaborate and nonahydrodecaborate have been synthesized starting from 2-chloro-10(3-bromopropyl)phenothiazine and ammonioundecahydrododecaborate or 2-ammoniononahydrodecaborate, respectively.

The so-called boron neutron capture therapy aims to destroy tumor cells by the following nuclear reaction,

$$^{10}_{5}B + {}^{1}_{0}n \longrightarrow {}^{7}_{3}Li + {}^{4}_{2}He + 2.79\ \text{MeV}\ (6.1\%)$$
$$\longrightarrow {}^{7}_{3}Li + {}^{4}_{2}He + 2.31\ \text{MeV}\ (93.9\%)$$
$$\longrightarrow {}^{7}_{3}Li + \gamma + 0.48\ \text{MeV}$$

while keeping intact the ordinary sound tissue or cells surrounding the tumor cells. The primary requisite for this therapy is to accumulate ^{10}B atoms selectively in or on tumor cells for a sufficient time. Chlorpromazine is known to bind selectively with melanin particles in melanoma cells.[2] The binding has been ascribed to the formation of a charge transfer complex between chlorpromazine (electron donor) and melanin (electron acceptor),[3] although positive-ion radicals produced from oxidation of chlorpromazine, especially photooxidation by white light, may play some role in the binding.[2,4,5] Irrespective of what is the nature of the binding, the neutron capture therapy may be applied to malignant melanoma, if a suitable boron derivative of chlorpromazine can be synthesized and administered to a patient without loss of this specific binding ability.



Ⅰ: X=BH_3, charge 0
Ⅱ: X=$B_{12}H_{11}$, charge 11
Ⅲ: X=$B_{10}H_9$, charge 11

Chart 1

A study along this line has been carried out by Mishima and collaborators[6] with chlorpromazineborane [I] supplied from the authors' laboratory.[7] The result was unsatisfactory probably owing to the water-insolubility and poor boron-content of [I], although weak inhibition was observed on tumor growth. To eliminate these disadvantages, the titled two compounds, or more strictly, N,N-dimethyl-N-[3-(2-chlorphenothiazin-10-yl) propyl]-amine-undecahydrododecaborate(1-) [II], and 2-{N,N-dimethyl-N-[3-(2-chlorphenothiazin-10-yl) propyl]amine}-nonahydrodecaborate-(1-) [III] have been synthesized.

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2) M.S. Blois, *J. Invest. Derm.*, **45**, 475 (1965).
3) A.M. Potts, *Invest. Ophthal.*, **1**, 522 (1962); *idem*, *ibid.*, **3**, 399 (1964).
4) L.H. Piette and I.S. Forrest, *Biochim. Biophys. Acta*, **57**, 419 (1962).
5) L.H. Piette, G. Bulow, and I. Yamazaki, *Biochim. Biophys. Acta*, **88**, 120 (1964).
6) Y. Mishima, 8th Int'l. Pig. Cell Conf.: Mechanisms in Pigmentation. Vol. 1, p. 215 (1973).
7) T. Nakagawa and Y. Mishima, Japan Patent 48-35024 (1973). [*C.A.* **79**, 361 115600 *U* (1973)].

These monovalent anionic compounds with high boron contents are water-soluble when paired with a suitable cation such as Na^+, stable in the absence of light, and obtainable from boron trifluoride. Because of the last advantage, a product of high ^{10}B content can be prepared easily. A preliminary report of Mishima on the medical aspects has shown a promising prospect[8] and further study is now in progress. The present paper describes only the method of syntheses.

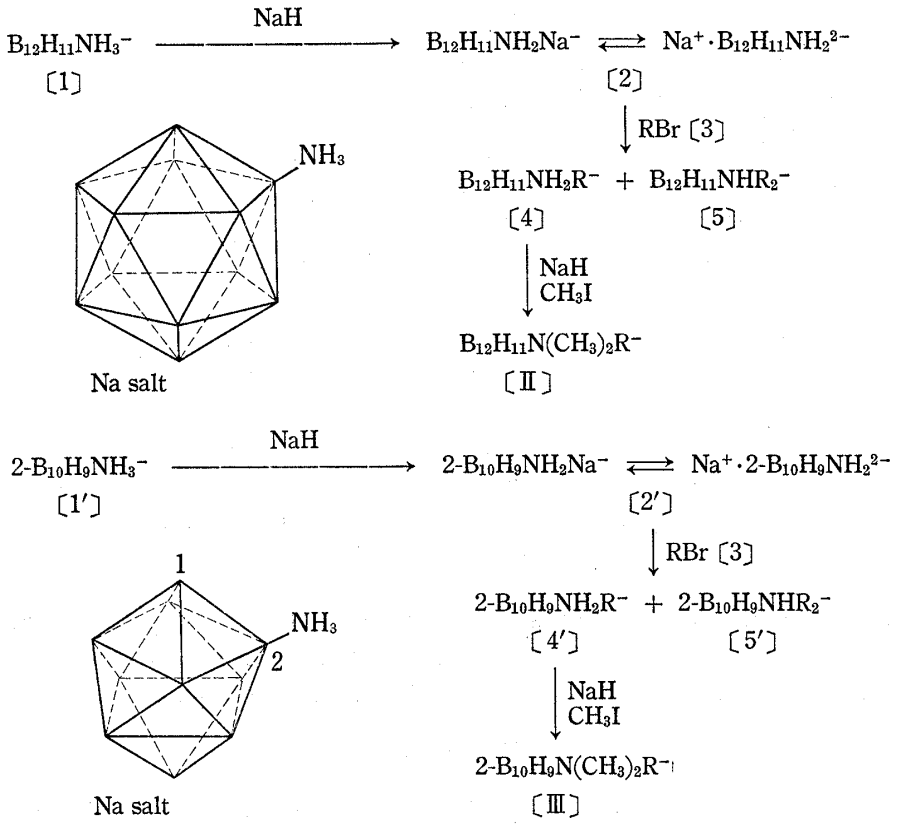
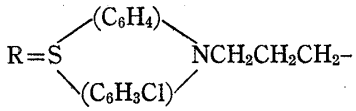


Fig. 1. The Routes of Syntheses



The routes of syntheses are illustrated in Fig. 1, where R stands for a 3-(2-chlorphenothiazin-10-yl)propyl group and the polyhedrons show the structures of starting materials, [1] and [1']; each apex is occupied by a boron atom accompanied with a hydrogen atom (not shown in the figure) or an NH_3 group.

Discussion

For obtaining pure crystalline products, the most important step is the isolation of [4] (or [4']) from the reaction mixture [4]+[5] ([4']+[5']), which is detailed in the experimental part. The correctness of chemical structures of both the products and the intermediates is supported by the course of synthesis, the elemental analysis, the infrared and especially nuclear magnetic resonance (NMR) data. Table I summarizes the NMR data of crystallized tetramethylammonium salts of the products, which can be transformed into medicinal sodium salts by the passage through a cation-exchanger of Na^+ type.

8) Y. Mishima, 38th Nihon Hifuka Gakkai Higashi-Nippon Rengo Chihokai, Special Lecture No. 6, p. 31.

Table I. Proton Magnetic Resonance (PMR) Data
(solv. CD_3CN, internal ref. tetramethylsilane)

$[(CH_3)_4N \cdot B_{12}H_{11}N(CH_3)_2R] \cdot (CH_3OC_2H_4OCH_3)_{0.75}$	τ	Relative intensity found	theo.
Benzene ring	2.93	7.0	7
α-CH_2	6.10	2.1	2
β-CH_2	(~7.8~	)	2
γ-CH_2	(6.73	2.0)	2
$-N(CH_3)_2-$	7.29	6.1	6
$^+N(CH_3)_4$	6.91	12.0[a]	12
$-OCH_3$	6.71	4.6	4.5
$-OCH_2$	6.54	3.1	3
$[(CH_3)_4N \cdot 2\text{-}B_{10}H_9N(CH_3)_2R] \cdot CH_3COCH_3$			
Benzene ring	2.95	7.0	7
α-CH_2	6.13	2.0	2
β-CH_2	(~7.8~	)	2
γ-CH_2	(6.85	1.9)	2
$-N(CH_3)_2-$	7.59	6.0	6
$^+N(CH_3)_4$	6.91	12.0[a]	12
$-COCH_3$	7.90	6.3	6

a) taken as 12. Data in parentheses have been obtained with hydrated sodium salts. The existence of partial structure $>N-\overset{\alpha}{C}H_2-\overset{\beta}{C}H_2-\overset{\gamma}{C}H_2-\overset{|}{N}-B$ was confirmed by PMR decoupling experiments.

The use of tetramethylammonium salts, in place of sodium salts, as the starting materials evoked some decomposition reactions and did not give the desired products. The attempted methylation of [4] by dimethyl sulfuric acid failed to produce [II].

Experimental

Starting Materials——$Na \cdot B_{12}H_{11}NH_3$, [1], and $Na \cdot [2\text{-}B_{10}H_9NH_3]$, [1′], were obtained by passing appropriate tetramethylammonium salts, which had been prepared by a conventional method,[9] through a cation-exchanger (Amberlite IR 120, Na^+ type) column. 2-Chloro-10-(3-bromopropyl)phenothiazine, [3], was synthesized by a method published by Imamura, *et al.*[10]

Reaction Mixture $B_{12}H_{11}NH_2R^-$+$B_{12}H_{11}NHR_2^-$, [4]+[5]——A solution comprising 2.5 g (14 mmoles) of [1] and 20 ml of dimethylformamide (DMF) was added dropwise under stirring to an ice-cooled suspension containing 490 mg of commercially available oil-coated sodium hydride (NaH content was *ca.* 50% *i.e.* 10.2 mmoles) in 3 ml of 1,2-dimethoxyethane. The amount of hydrogen gas evolved during the time of addition, 15 min, was about 11 mmoles. The reaction flask was then cooled to −10——5°, and 3.0 g (8.5 mmoles) of [3] dissolved in 15 ml of DMF was added dropwise in 20 min. After slow elevation of reaction temperature to 15°, the stirring was continued for 2 hr. The reaction mixture was poured into 300 ml of dried ether. The resulted gummy precipitate was collected and dissolved in 500 ml of water followed by the neutralization with dilute sulfuric acid. The addition of excess tetramethylammonium chloride to this aqueous solution precipitated a mixture of tetramethylammonium salts of [4] and [5].

Isolation of $B_{12}H_{11}NH_2R^-$, [4]——The salt mixture was once dissolved in acetonitrile, dried by evaporating off the acetonitrile, and redissolved in a mixture of acetone (5 ml) and 1,2-dimethoxyethane (50 ml). Evaporation of acetone crystallized out 2.5 g of tetramethylammonium salt of [4] containing one mole of 1,2-dimethoxyethane as a crystallization solvent. The precipitate was filtered off from a filtrate which contained [5], treated again with the acetone–dimethoxyethane mixture, and finally recrystallized from acetone. This product had no crystallization solvent after drying at 120°. Yield 1.8 g, 42%. *Anal.* Calcd. for $C_{19}H_{38}N_3SClB_{12}$: C, 45.1; H, 7.6; N, 8.3; S, 6.3; Cl, 7.0. Found: C, 45.2; H, 7.9; N, 8.3; S, 6.7; Cl, 7.0.

$B_{12}H_{11}N(CH_3)_2R^-$, [II]——The product (1.4 g, 2.77 mmoles) dissolved in 20 ml of DMF was added dropwise to an ice-cooled suspension of sodium hydride (5.5 mmoles, 266 mg of 50% NaH suspended in 1,2-dimethoxyethane) with stirring. Dropwise addition of methyl iodide (860 mg, 6 mmoles in 2 ml of DMF) caused the

9) W.R. Hertler and M.S. Raasch, *J. Am. Chem. Soc.*, **86**, 3661 (1964).
10) H. Imamura, T. Okada, E. Matsui, and Y. Kato, *Yakugaku Zasshi*, **90**, 813 (1970).

precipitation of tetramethylammonium iodide. After 20 min, sodium hydride suspension (5.5 mmoles) and methyl iodide (6 mmoles) were supplemented. The reaction mixture was filtered after stirring for 2 hr. Crude tetramethylammonium salt of [II] was precipitated by pouring the filtrate into an aqueous solution of tetramethylammonium chloride. The crude salt was collected, dried, and treated twice with the acetone–dimethoxyethane mixture in a similar way as described in the preceding paragraph. The product thus purified had 0.75 mole of 1,2-dimethoxyethane as a crystallization solvent. Yield 1.5 g, 90%; mp 159—161°. *Anal.* Calcd. for $C_{24}H_{49.5}N_3SClO_{1.5}B_{12}$: C, 47.9; H, 8.3; N, 7.0; S, 5.3; Cl, 5.9. Found: C, 48.0; H, 8.5; N, 7.2; S, 5.2; Cl, 6.0.

The tetramethylammonium salt could be converted to sodium salt by charging it to a cation exchanger (Amberlite IR 120, Na^+ type) column thoroughly washed with an acetonitrile (1)–water (1) mixture, and eluting with the same mixture in the dark. Concentration of the effluent to dryness below 40° gave the sodium salt without significant loss.

Reaction Mixture $B_{10}H_9NH_2R^-$+$B_{10}H_9NHR_2^-$, [4′]+[5′]——The mixture of tetramethylammonium salts was prepared from 2.5 g of [1′], 0.37 g of NaH (50%), and 2.80 g of [3] by a quite similar method as used for obtaining dodecaborate homolog mixture [4]+[5].

Isolation of $B_{10}H_9NH_2R^-$, [4′]——A preliminary experiment showed the inability of the acetone–dimethoxyethane treatment to isolate [4′] from the reaction mixture. It is well known that phenothiazine derivatives often form micelles in aqueous solutions likewise most surfactants do,[11] and that the gel filtration chromatography is a powerful isolation method for a mixture of surfactants.[12] The following chromatography has been applied with success to the isolation of [4′].

The gegen-ion of the mixture, [4′]+[5′], was converted from tetramethylammonium to sodium by the same procedure as used in the similar conversion of [II]. The sodium salt mixture thus obtained was dissolved in 8 ml of water, charged to a gel column soaked with water (Sephadex G-25 medium Pharmacia; bed vol. 200 ml; void vol. estimated by Blue Dextran 80 ml), and eluted with water. The 80—362 ml fraction contained almost pure [5′], the 850—2000 ml fraction was rich in [4′], and the 2000—2400 ml fraction was rich in [5′] probably owing to the desorption of [5′] promoted by the migration of partner component [4′].[12]

The middle fraction was concentrated, and after addition of acetone, dried to give a residue still including 20—30% acetone. The ratio of [4′]: [5′] was estimated to be 90—95: 10—5 by the NMR spectrum which also revealed the inclusion of a minute amount of impurity other than [5′]. The residue was dissolved in 1,2-dimethoxyethane, and dried *in vacuo* to give 1.2 g of sodium salt containing 1.5 mole of 1,2-dimethoxyethane as a crystallization solvent. Yield *ca.* 27%.

$B_{10}H_9N(CH_3)_2R^-$, [III]——In a quite similar way as in the case of [II], crude tetramethylammonium salt of [III] was prepared by the reaction of 1.1 g of the product, 0.21×2 g of 50% sodium hydride, and 0.60×2 g of methyl iodide, followed by the precipitation with tetramethylammonium chloride. Recrystallization from acetone and 2-hr drying *in vacuo* at room temperature gave pure tetramethylammonium salt of [III] which contained 1.0 mole of acetone as a crystallization solvent. Yield 0.88 g, 78%. *Anal.* Calcd. for $C_{24}H_{46}ON_3SClB_{10}$: C, 50.7; H, 8.2; N, 7.4; S, 5.6; Cl, 6.2. Found: C, 51.4; H, 8.4; N, 7.5; S, 5.8; Cl, 6.3. The conversion to sodium salt was as easy as that for [II].

11) D. Attwood, A.T. Florence, and J.M.N. Gillan, *J. Pharm. Sci.*, **63**, 988 (1974).
12) T. Nakagawa and H. Jizomoto, *J. Am. Oil Chemists' Soc.*, **48**, 571 (1971).