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Sucrose in Methanolic Calcium Chloride

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The solubility of sucrose in methanolic calcium chloride solution and the effect of various parameters were studied. The solubility of sucrose in methanol containing 30% $CaCl_2$ (w/v) was 45% (w/v) at 68 °C. Addition of acetone to the system recovered approximately 90% of the sucrose as a precipitate. Precipitation with phosphoric acid removed most of the calcium as tricalcium phosphate, leaving sucrose in solution. Methanolic NaOH precipitated both the sucrose and calcium in methanol, while with aqueous NaOH formation of the sucrose–$Ca(OH)_2$ complex depended on the concentration of the reactants.

Complexes of carbohydrates with alkali and alkaline-earth metal salts and bases in aqueous solution have been extensively studied (Jensen et al., 1940; Rendleman, 1966a; Roy and Mitra, 1972; Moulik and Mitra, 1973; Moulik and Khan, 1975). The interaction of CaO with sucrose has long been utilized in the Steffen process in the beet sugar industry (McGinnis, 1982). Similar procedures have been the subject of extensive investigations in the dairy industry in efforts to recover lactose from cheese whey (Cerbulis, 1973; Nickerson, 1979; McCommins et al., 1980; Quickert and Bernhard, 1982). However, investigations of alkali-metal complexes of carbohydrates in alcoholic solutions are rare, except for the comprehensive study by Rendleman (1966b,c) on the interaction, in ethanol, of a series of sodium and potassium salts and bases with carbohydrates and their derivatives. In previous studies of nonaqueous solvents for carbohydrates, methanol has received little attention due to its relatively low solvent power (less than 1% for sucrose) (Moye, 1972). Domovs and Freund (1960) observed increased solubility for a wide range of carbohydrates in methanol containing calcium chloride, described a heptahydrate complex of lactose–calcium chloride in the presence of water, and obtained a crystalline lactose–$CaCl_2$–$4CH_3OH$ under anhydrous condition. A sodium hydroxide complex of sucrose has been isolated from methanolic media by Rendleman (1966c).

The objectives of the present study were to (1) determine the effects of various parameters such as temperature and concentration on the solubility of sucrose in methanolic calcium chloride solution and (2) investigate possible methods to recover the sucrose from the system.

EXPERIMENTAL PROCEDURES

Determination of Solubility. Methanolic calcium chloride stock solution was prepared by saturating methanol (0.02% moisture) with anhydrous $CaCl_2$ by shaking for 1 h at 60 °C. Solutions of various concentrations of calcium chloride were then prepared by diluting the stock solution. All experiments were done in capped 25-mL vials. The solubility of sucrose in methanol at various concentrations of $CaCl_2$ was determined by adding sufficient sucrose to always form an equilibrium of saturated sucrose solution and sucrose crystals at a given temperature and time of mixing.

Precipitation of Sucrose and Calcium from Solution. Fixed volumes of various concentrations of sodium hydroxide, phosphoric acid, and acetone were added to the methanol solution of sucrose and $CaCl_2$ with rapid mixing, in 12-mL clinical centrifuge tubes. The flocculent precipitate formed was set for 10 min and then centrifuged down. The supernatant was analyzed for sucrose, calcium, and chloride concentrations. A methanolic NaOH solution was prepared and added to the methanolic $CaCl_2$–sucrose solution. Alternatively, the methanolic $CaCl_2$–sucrose solution was evaporated to dryness, before an aqueous solution of NaOH was added. In all recovery experiments, a 4–6% sucrose solution was used. The concentration of sucrose remaining in solution was calculated on the basis of the initial volume of the methanolic $CaCl_2$–sucrose solution used.

Carbohydrate Analysis. The sucrose dissolved in methanolic calcium chloride solution was analyzed by the phenol–H_2SO_4 method described by Dubois et al. (1956). The methanol in the sample did not interfere with the colorimetric determination. In some cases, to detect the decomposition of sucrose, methanol was evaporated and the ferricyanide submicro method was used (Guinn, 1967).

Calcium Determination. Aliquots of sucrose-containing methanolic $CaCl_2$ solution were air-dried before 7.5 mL of hydroxynaphthol blue (HNB) buffer solution (pH 13.0) and 42.5 mL of H_2O were added. The solution was titrated with standardized titraver solution ($^1/_{28}$ N EDTA, pH 5) to a blue end point using 0.1 g HNB as indicator (Zaragosa et al., 1982).

Alternatively, calcium was analyzed by atomic absorption using a Perkin Elmer 303 AA spectrophotometer on samples prepared by dry ashing (Anon 1982).

Chloride Determination. The Mohr method by titrating with $AgNO_3$, using K_2CrO_4 as an indicator, was used (Johnson and Ulrich, 1959). In all the determinations, the methanol was evaporated before analyses.

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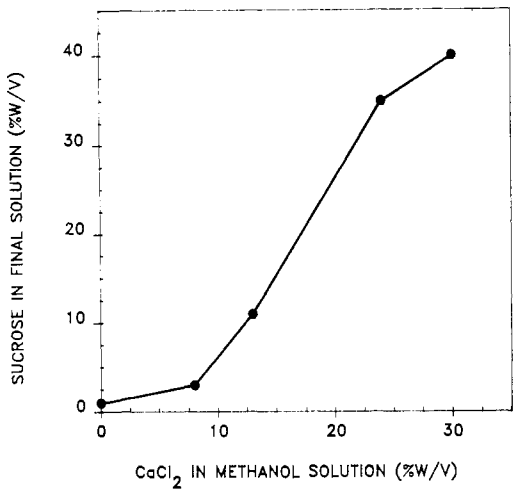

Figure 1. Effect of calcium concentration on solubility of sucrose. Conditions: solid sucrose (50% w/v) mixed with $CH_3OH/CaCl_2$ solution, heated at 60 °C for 1 h.

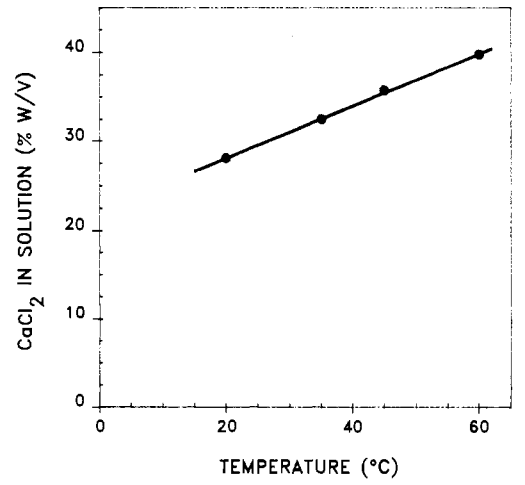

Figure 2. Solubility of calcium chloride in methanol. Conditions: $CaCl_2$ mixed with CH_3OH, heated for 1 h at various temperatures.

HPLC Analysis. To monitor changes of sucrose following some treatments, the product was analyzed by HPLC. The liquid chromatograph consisted of a Spectra Physics SP8700 solvent delivery system with a SP6040 differential refractometer, an SP4270 integrator, and an Aminex Carbohydrate HPX-87C column (250 × 4 mm, Bio-Rad). Samples were evaporated to dryness, redissolved in H_2O to the appropriate concentration, and filtered through a 0.45-μm filter (Prep-Disc, Bio-Rad) before analysis. Appropriate concentrations of standard solutions of sucrose, glucose, and fructose were prepared and used for quantitative calculations. The eluate was H_2O with a flow rate of 0.3 mL/min. Column temperature was maintained at 85 °C with a column heater (Eldex).

RESULTS AND DISCUSSION

Methanolic Calcium Chloride Solution. The solubility of sucrose in methanol was found to be dependent upon the amount of $CaCl_2$ in solution and the temperature. The solubility was increased approximately 40-fold with 30% $CaCl_2$ in methanol (w/v) at 60 °C as compared to the solubility in methanol alone (Figure 1). The concentration of $CaCl_2$ used was restricted by its solubility in methanol at a given temperature. Figure 2 illustrates that the solubility of $CaCl_2$ in methanol increased from 28% (w/v) at 20 °C to 40% at 60 °C. The effect of temperature on the solubility of sucrose in methanolic $CaCl_2$ solution is shown in Figure 3. With 25% of $CaCl_2$, the solubility increased

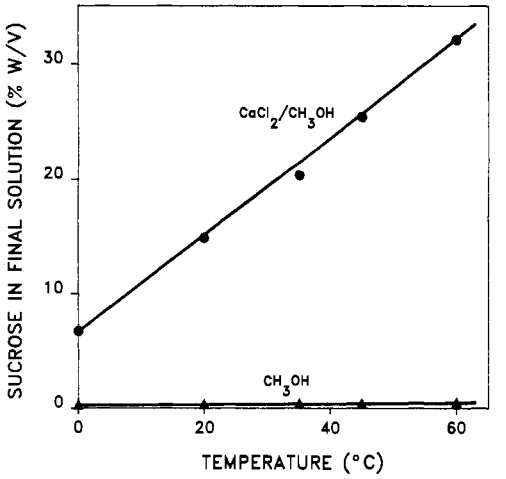

Figure 3. Effect of temperature on solubility of sucrose in methanolic calcium chloride solution. Conditions: solid sucrose (50% w/v) in $CH_3OH/CaCl_3$ (25% w/v) solution, heated at 20, 35, 45, and 60 °C for 1 h.

4.5 times (7–32%) when the temperature was raised from 0 to 60 °C. (A 45% solubility could be achieved at a reflux temperature of 68 °C.) In methanol alone, over the temperature range studied, the solubility of sucrose remained constant at about 1%.

Under the same conditions, the solubility of sucrose in ethanol or ethanolamine was not increased by the addition of $CaCl_2$. Experiments were conducted using a number of other calcium and magnesium salts and bases in methanol. Of the following investigated ($CaHPO_4$, $CaCO_3$, CaO, MgO, $MgCl_2$, $MgSO_4 \cdot 7H_2O$), only $MgCl_2$ and $MgSO_4$ caused a slight increase in the solubility of sucrose as compared to the control. The greatest increase obtained was about 20% of that observed with $CaCl_2$. This is mainly due to the fact that these compounds were insoluble or slightly soluble in methanol. Additional experiments were done in which $Ca(OH)_2$ was converted to $Ca(HS)_2$ in methanol by bubbling H_2S through a suspension of $Ca(OH)_2$ in methanol. The resulting 15% solution of $Ca(HS)_2$ in methanol was able to dissolve sucrose readily (17% w/v at 30 °C). This indicates that the effectiveness of the system in dissolving sucrose depends on the amount of metal salts in solution. The solvent power of alcohols for salts decreases with increasing carbon number. In general, metals salts are more soluble in methanol than in ethanol (Lloyd et al., 1928; Rendleman, 1966b).

Sodium and potassium salts in ethanol have been shown to increase the solubility of carbohydrates (Rendleman, 1966b), although the increase was relatively small compared to that observed in the system used in the present study. The salts possibly caused a decrease in hydrogen bonding between carbohydrate molecules, allowing more metal cations to be attached to the molecules. A complex between lactose and $CaCl_2$ in methanol has been shown to exist in the molar ratio of unity (Domovs and Freund, 1960). In ethanol, potassium ion has been shown to bind sucrose in a 2:1 molar ratio. The ratio seems to vary depending on the cation concentration, the ionic radii, and the configuration of the carbohydrate molecule (Rendleman, 1966b,c).

Stability of Sucrose in Methanolic Calcium Chloride. Sucrose was found to be very stable in methanolic $CaCl_2$ solution. Analyses by HPLC of samples after 12 days of storage under room temperature showed no changes. Sucrose in 90% ethanol has been shown to degrade considerably at a temperature of 78.5 °C (Simizu et al., 1962). The stability of sucrose may be attributed to the soluble complex formed between sucrose and $CaCl_2$ in

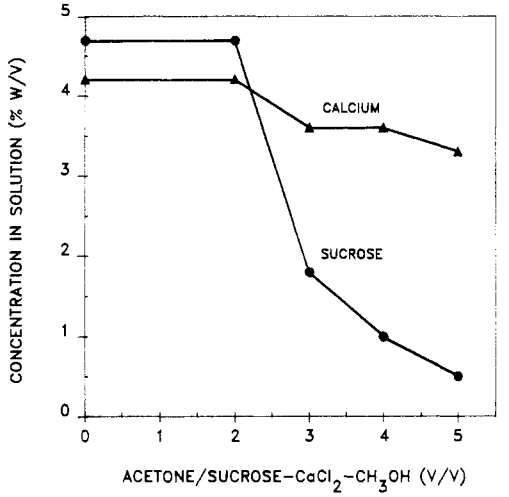

Figure 4. Precipitation of sucrose with acetone.

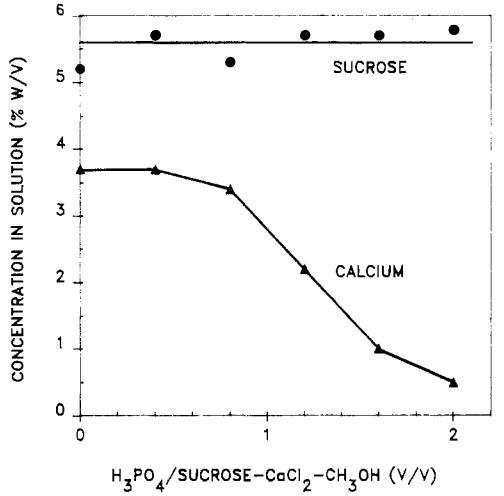

Figure 5. Precipitation of calcium with phosphoric acid.

methanol. The dissociation of sucrose from $CaCl_2$ in methanol required more drastic conditions than that of the lactose complex which could be separated by introducing water or methanol to the system (Domovs and Freund, 1960).

Acetone Precipitation of Sucrose. Organic solvents such as ether and ethyl acetate have been shown to decrease the solubility of alkali-metal complexes of carbohydrates in ethanol (Rendleman, 1966a). Of the common solvents tried in this study, only acetone separated the sucrose as a precipitate from the methanolic $CaCl_2$ solution. Addition of acetone precipitated the sucrose while leaving most of the $CaCl_2$ in solution. Approximately 90% of the sucrose could be recovered rapidly by using a 5:1 ratio of acetone to solution as shown in Figure 4. This procedure was nondestructive and allowed possible recycling of the methanolic $CaCl_2$ solution after removing the acetone. A similar approach has been employed by Olano et al. (1977), using 20% acetone to precipitate the lactose–$Ca(OH)_2$ complex from aqueous solution.

Phosphoric Acid Precipitation of Calcium. Phosphoric acid complexes with calcium, leading to the precipitation of tricalcium phosphate. Almost complete removal of the calcium was achieved, leaving sucrose in the solution (Figure 5). The interaction of calcium and phosphoric acid to form tricalcium phosphate is the principal reaction of the phosflotation process used in certain part of sugar industry for removal of impurities from diffusion juice. Inversion of sucrose in the process was estimated to be 0.01% (Saranin, 1972). However, the

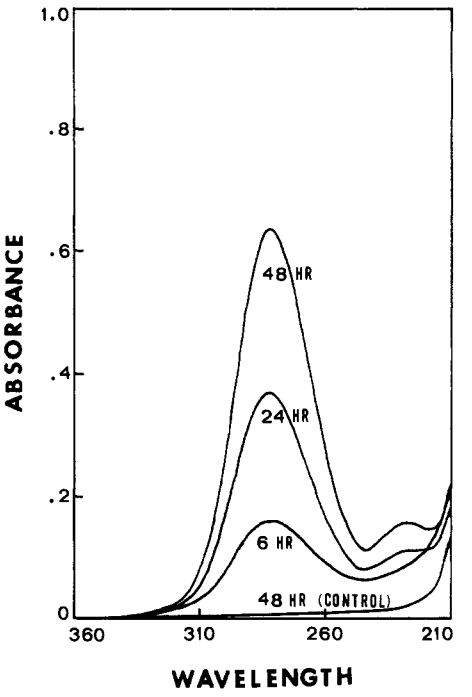

Figure 6. Ultraviolet spectra of phosphoric acid treated methanolic calcium chloride solution of sucrose.

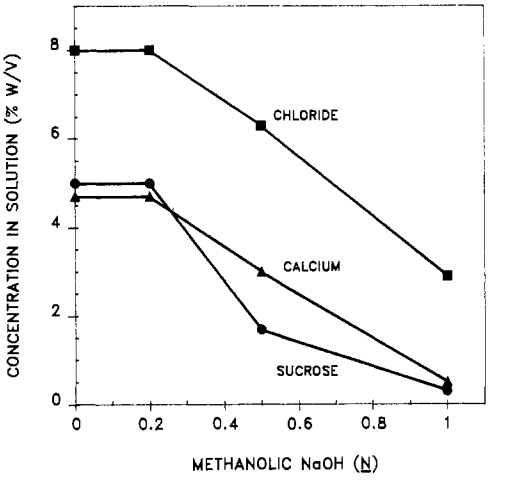

Figure 7. Addition of methanolic sodium hydroxide solution. CH_3OH–NaOH:sucrose–$CaCl_2$–CH_3OH = 2:1 (v/v).

changes of sucrose in methanolic H_3PO_4 solution are not known. Precipitation of calcium from the methanolic solution by phosphoric acid was slow in comparison to the acetone precipitation of sucrose. Furthermore, the precipitate gradually redissolved, and the solution discolored with time. The color formation may be due to the acid catalyzed hydrolysis of sucrose and the conversion of the monosaccharides to 5-(hydroxymethyl)furfural and its decomposition products. The dissolved salts in the solution also contribute to the acceleration of the hydrolysis (Feather and Harris, 1973). The UV spectrum of the phosphoric acid treated methanolic $CaCl_2$ solution of sucrose was monitored at time intervals and compared with control samples in which the phosphoric acid was replaced by water (Figure 6). The development of absorption peaks at 230 and 283 nm and their increasing intensity with time suggest the formation of furfural and other color precursors following the hydrolytic degradation of the sucrose (Parker and Williams, 1968).

Sodium Hydroxide Precipitation of Sucrose and Calcium. Adding methanolic NaOH solution directly to the sucrose solution precipitated both sucrose and $CaCl_2$

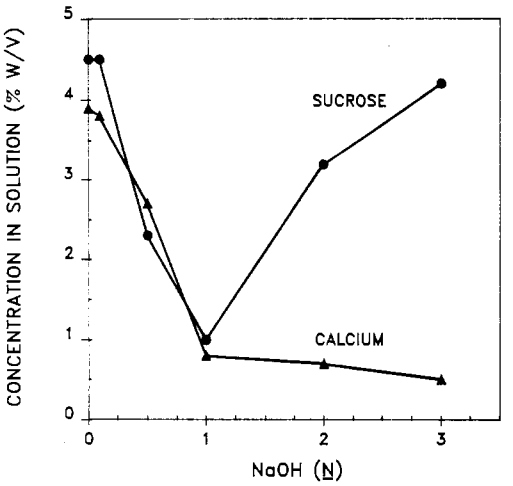

Figure 8. Addition of aqueous sodium hydroxide solution. Conditions: aqueous NaOH:sucrose–$CaCl_2$–CH_3OH = 2:1 (v/v), sucrose/$CaCl_2$/CH_3OH air-dried before adding NaOH solution.

from the system (Figure 7). The stoichiometry ratio of calcium to chloride in the precipitate was approximately 2:1. In previous work with anhydrous ethanol, hydroxides of alkali metals react with sucrose to form precipitates that are mostly monoalcoholate (Rendleman, 1966c). However, in the present system of sucrose in methanolic calcium chloride solution, the precipitate also contains NaCl since the solubility of the sodium salt is exceedingly low in methanol (Lloyd et al., 1928; Moye, 1972).

In aqueous NaOH solution, the precipitation of sucrose was dependent on the concentration of NaOH (Figure 8). At a low concentration ratio of NaOH to $CaCl_2$ (2:1, w/w), the sucrose was coprecipitated with calcium. In analogous experiments done by Vandewijer et al. (1972), almost quantitative sucrose yields were obtained by adding NaOH slowly to aqueous sucrose solutions containing the required amount of $CaCl_2$; the $Ca(OH)_2$ that was formed in situ, unlike hydrated lime added to the medium, was able to precipitate the sucrose.

The glycosidic linkage of sucrose is more stable in alkaline than in acidic medium, but considerable decomposition has been known to occur at high temperature and pH (Mauch, 1971). Analyses of the supernatant by HPLC showed that little decomposition of sucrose occurred during the NaOH treatment used in the present experiment (Figure 9).

When the concentration ratio of NaOH to $CaCl_2$ was increased (6:1, w/w), 96% of the sucrose remained in solution and all the calcium precipitated as $Ca(OH)_2$. Olano et al. (1977) used a similar approach to precipitate lactose–$Ca(OH)_2$ complex from an aqueous solution of $CaCl_2$ and lactose and found that by adding high enough concentration of NH_4Cl the lactose–$Ca(OH)_2$ complex was solubilized. It is likely that, in the present study, a high concentration ratio of NaOH to $CaCl_2$, with formation of excess NaCl, would solubilize the complex by a similar mechanism explaining the fact that most sucrose was in solution in samples with an initial high concentration ratio of NaOH to $CaCl_2$.

The chemistry and technological applications of carbohydrates have been largely limited by their general low solubility in organic solvents. The many interactions of carbohydrates with other components in systems such as food are highly complex and seldom studied. The present report describes the complexation of sucrose in methanolic $CaCl_2$ solution, the resulting enormous increase in the solubility of sucrose, and the reversibility of the complexation for sucrose. The results obtained in this investigation are currently being tested on the extraction and recovery of sucrose from a multicomponent system.

Figure 9. Sucrose in aqueous sodium hydroxide treated samples: A, 1 N NaOH; B, 3 N NaOH.

Registry No. $CaCl_2$, 10043-52-4; $MgCl_2$, 7786-30-3; $MgSO_4$, 7487-88-9; $Ca(HS)_2$, 12133-28-7; Me_2CO, 67-64-1; H_3PO_4, 7664-38-2; NaOH, 1310-73-2; MeOH, 67-56-1; sucrose, 57-50-1.

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Structural Requirements for Bridged Bicyclic Compounds Acting on Picrotoxinin Receptor

Yoshihisa Ozoe and Fumio Matsumura*

To study essential requirements of cyclodiene type chemicals to interact with the picrotoxinin receptor, several cyclic hydrocarbons were synthesized. 8-Isopropylidenebicyclo[3.2.1]oct-6-en-3-one derivatives with equatorial *cis*-2,4-dimethyl substituents were found to be toxic to the German cockroach. By contrast, the axial cis and trans isomers were nontoxic. Some derivatives of bicyclo[2.2.1]heptene were moderately toxic. The endo cyclic sulfite of 5,6-bis(hydroxymethyl)bicyclo[2.2.1]hept-2-ene was most toxic. The cyclodiene-resistant strain of the German cockroach exhibited cross-resistance to representative bridged bicyclic compounds. These compounds also inhibited specific [^{3}H]-α-dihydropicrotoxinin binding to the American cockroach brain membrane components. The potency of *endo*-5,6-bis(chloromethyl)-7-isopropenylbicyclo[2.2.1]heptan-2-one was comparable to those of cyclodiene insecticides. These observations suggest that these insecticidal bridged bicyclic compounds act at the picrotoxinin binding site. There appears to be a minimum requirement for an active ligand to possess at least two of the three active sites: two electronegative and one steric bulkiness (hydrophobicity) centers.

INTRODUCTION

γ-Aminobutyric acid (GABA) is regarded as an inhibitory neurotransmitter in a variety of animals. The mechanism of GABAergic synaptic transmission is rapidly being elucidated at subcellular levels. The GABA system in the central nervous system (CNS) of mammalian species consists of at least three closely coupled components, i.e., the GABA receptor, the chloride ionophore, and the benzodiazepine recognition site (Ticku, 1983; Bowery, 1984). γ-Aminobutyric acid released from the nerve terminal binds to the GABA receptor on the postsynaptic membrane. The binding causes an increase in the membrane permeability to chloride. A naturally occurring convulsant, picrotoxinin, is known to antagonize the action of GABA by blocking the ionophore (Takeuchi and Takeuchi, 1969; Ticku et al., 1978).

Recently, Kadous et al. (1983), Matsumura and Ghiasuddin (1983), and Tanaka et al. (1984) have presented the evidence that cyclodiene insecticides and γ-BHC compete with picrotoxinin at a common binding site in the cockroach brain. As a result they have concluded that their interaction with the picrotoxinin receptor plays an important role in their convulsive action in insect CNS. This conclusion is based on the following evidence. The cyclodiene-resistant strains of the German cockroach show cross-resistance to picrotoxinin. Cyclodiene-type insecticides, including γ-BHC, inhibit the specific binding of [^{3}H]-α-dihydropicrotoxinin (an active analogue of picrotoxinin) at the picrotoxinin receptor. The neurophysiological effect of picrotoxinin was similar to that of cyclodiene-type insecticides.

In addition, Matsumura and Ghiasuddin (1983) noticed structural similarity among picrotoxinin, heptachlor epoxide, and γ-BHC (Figure 1). The first two compounds have bridged bicyclic structure: 6-oxabicyclo[3.2.1]octan-7-one for picrotoxinin; bicyclo[2.2.1]heptene for heptachlor epoxide. Two rings at the 1-, 2-, and 6-positions of picrotoxinin may correspond to the epoxycyclohexane ring of heptachlor epoxide and three equatorial chlorines of γ-BHC. γ-BHC does not have any bridged bicyclic structures but two axial chlorines that may correspond to the γ-lactone ring. Also the central axial chlorine of γ-BHC has the same orientation as the isopropenyl group of picrotoxinin.

Structure–insecticidal activity relationship of picrotoxinin analogues and related compounds has been studied by several researchers (Miller et al., 1979; Kuwano et al., 1980), who noticed that the bridged bicyclic lactone skelton and the *trans*-isopropenyl or isopropyl group are essential for insecticidal activity. Structure–activity relationship of cyclodiene insecticides and BHC has also been thoroughly discussed (Soloway, 1965; Brooks, 1973, 1974). However, these discussions were held before the realization of the nature of their biological target site(s).

In view of the recent discovery of similarities of action patterns between picrotoxinin and cyclodiene-type insecticides as described above (cf., Miller et al., 1979; Matsumura and Tanaka, 1984), it appears worthwhile to reexamine structural requirement of picrotoxinin-type convulsants for interaction with the specific picrotoxinin binding site. Another objective of this study is to obtain supporting evidence for the role of the picrotoxinin receptor in the mode of action of cyclodiene-type insecticides by synthesizing compounds that structurally bridge the gap between cyclodiene compounds and picrotoxinin.

MATERIALS AND METHODS

Chemicals. Picrotoxinin and picrotin were separated from commercially available picrotoxin with silica gel column. Camphor, norcamphor, and norbornylene were provided by Aldrich Chemical Co. α- and β-endosulfan were supplied by the Environmental Protection Agency. Alodan was synthesized by lithium aluminum hydride reduction of the Diels–Alder adduct between penta-

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