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Technical Notes

Isolation and Identification of the Major Extractable Impurity from Dowex 1×2 Resin

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Abstract:

Structurally related low-molecular weight organic molecules which accounted for ~0.13% of the total mass were isolated from commercial grade Dowex 1×2 resin. The structure of the main impurity is 2-chloromethyl-3-methoxyl-1-phenyl-1-propanol. This major component and the other extractables were the reaction products of monomeric styrene and chloromethyl methyl ether used in the production of Dowex 1×2 resin. These impurities were most likely entrapped in the polymer matrix and not sufficiently removed during the washing and cleaning process steps.

Introduction

Dowex 1×2 resin is a synthetic styrene−divinylbenzene copolymer containing quaternary ammonium functional groups.[1,2] It is the key ingredient of a prescription drug under the trade name of Questran, commonly used for cholesterol control. However, the major use of Dowex 1×2 is as a strongly basic anion-exchange resin used in chromatography.

Chromatography resins should not only provide selectivity for ion exchange, but also be of high quality. Often, contaminants that originated from resins have been found in the process streams, thus complicating the purification efforts.[3,4] The most commonly found contaminations from chromatographic media are the leakage of the media itself or contaminating small monomeric organics from the manufacturing process. To ensure successful process chromatography, potential contamination due to the resin should not be overlooked; thus, the identities of the residual compounds have to be characterized. According to the Code of Federal Regulation (CFR), the organic extractables in a polymer ion-exchange resin must not exceed 1 ppm.[5] This report describes the isolation of several structurally related organic extractables from commercial grade Dowex 1×2 resin. The major extractable was further purified, and its chemical structure was fully characterized using nuclear magnetic resonance (NMR) and mass spectrometry (MS).

Results and Discussion

Isolation. An array of commonly used solvents was evaluated for extractability of Dowex 1×2, and methylene chloride was chosen due to its excellent swelling and extraction properties for this resin. Typically, extraction of 60 g of commercial grade Dowex 1×2 resulted in isolation of ca. 80 mg of oily residue (0.13%, w/w) when extracted with methylene chloride.[6] Analysis of the crude extract by gas chromatography (GC) using a nonpolar DB-1 capillary column resulted in eight components being detected with only one major peak corresponding to ca. 95% of the total ion current. From this crude extract, eleven components were detected by reversed-phase high-performance liquid chromatography (RP-HPLC) using a Vydac C18 column. The largest component that represented ca. 70% of the total isolated extractables was further purified using semipreparative RP-HPLC for structure characterization.

However, the presence of these small extractables in the resin becomes insignificant if proper pre-washing of the resin is performed. The manufacturer of Dowex 1×2 resin recommends pre-washing steps using hydrochloric acid and sodium hydroxide prior to resin use.

Characterization. Gas chromatography/mass spectrometry analyses showed that most of the components had a base peak of *m*/*z* 107 as does the major component. This implied that these extractables are structurally related molecules. The major component exhibited a molecular ion of *m*/*z* 214 along with a strong P+2 peak at *m*/*z* 216, corresponding to a possible chlorine isotope peak under electron impact mass spectrometry (EI/MS) and electrospray mass spectrometry (ES/MS) conditions. Bis-trifluoroacetamide (BSTFA) derivatization of the crude extract suggested only one silylation site was available on the major component, and it was located on the base peak fragment.

The ^{1}H NMR spectra of the crude extract and purified major component (Figure 1) were virtually identical, indicating structural similarity between various components in this

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(1) Mindick, M.; Svarz, J. U.S. Patent 3,549,562, 1970.

(2) *The complete drug reference: United States Pharmacopeia* [United States Pharmacopeial Convention, Inc.]; Consumer Reports Books: Yonkers, NY, 1990; Vol. XXII, p 304.

(3) Johansson, B.-L. *BioPharm*. **1992**, 34−37.

(4) Knight, P. *Bio/Technology* **1989**, *7*, 777−782.

(5) The pretreatment procedure was recommended by manufacturer Dow Chemicals to meet the CFR test requirements. U.S. Food, Drug, and Cosmetic Act Regulation Number CFR 173.25.

(6) This was based on three batches of Dowex 1×2 resin available during the time of the studies.

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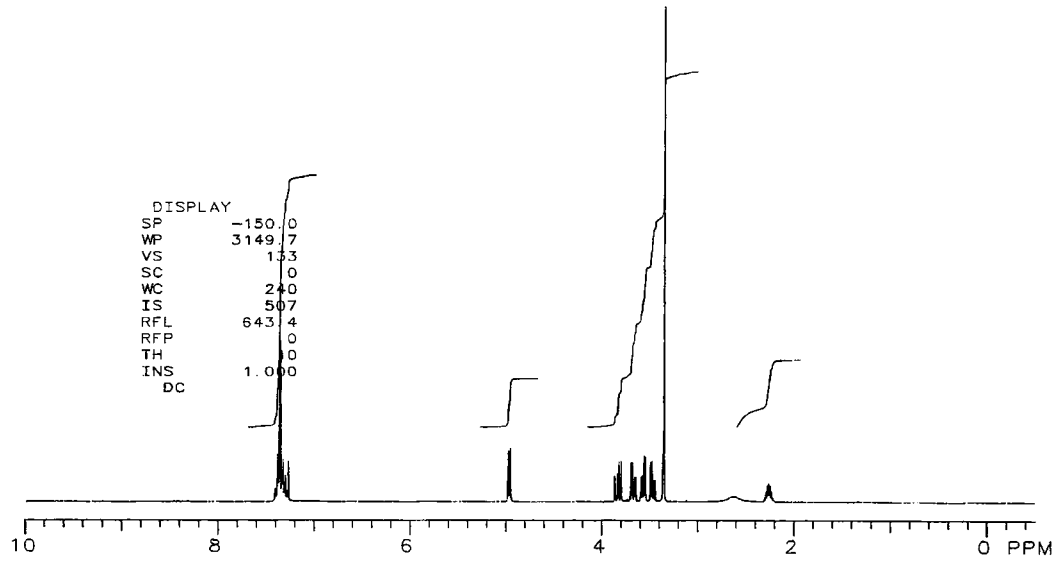


Figure 1. **Proton NMR spectrum of the purified major component 1.**

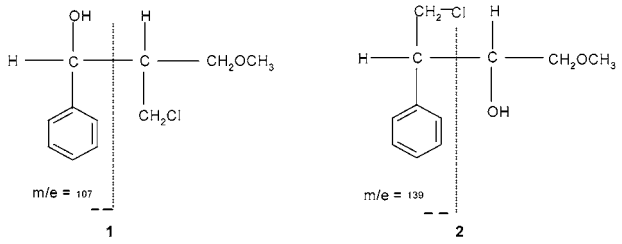


Figure 2. **According to ^{1}H NMR, two possible structures were considered for the major component. However, only structure 1 (but not structure 2) could yield a base peak of *m*/*z* 107.**

extractable. Initial examination of the ^{1}H NMR spectrum led to the suggestion that this extractable was a monosubstituted styrene derivative. The multiple peaks at 7.3 ppm represented five aromatic protons. The singlet at 3.4 ppm could be the three methoxy protons (see below). The multiplets at 3.4−3.9 ppm have a well-defined splitting pattern that accounts for two pairs of methylene protons. The one proton between 2.5 and 3 ppm was proven to be the hydroxyl proton as evidenced by a deuterium exchange experiment (data not shown).

Two possible chemical structures, both having a monosubstituted aromatic ring, were originally proposed for this major component (see **1** and **2** in Figure 2). To determine the positions of −OH and −CH_2Cl based on the ^{1}H and ^{13}C NMR analyses was not quite obvious. However, the characteristic base peak of *m*/*z* 107 in the EI/MS spectra provided an unambiguous assignment of a benzyl alcohol moiety. This ruled out structure **2** shown at right in Figure 2 that would result in a base peak of *m*/*z* 139 instead.

As discussed below, the proposed structure **1** is fully supported by other spectroscopic techniques such as ^{1}H NMR proton−proton decoupling (Figure 3) and ^{13}C NMR. Attached Proton Test (APT) NMR experiment, typically used to determine the number of protons directly bonded to a carbon, provided a secondary confirmation for the assigned structure. In addition, high-resolution mass spectrometry under FAB/MS conditions is also consistent with the proposed molecular formula of $C_{11}H_{15}O_2Cl$.

The assignments of the two vicinal protons (H_a and H_b) and the four methylene protons (* and **) can be established due to the uniqueness of the ^{1}H NMR coupling pattern (Figure 3). A proton−proton decoupling experiment with the irradiation of proton H_b at 2.3 ppm resulted in a singlet peak of proton H_a at 5.0 ppm, while the four pairs of doublet of doublets at 3.4−3.8 ppm collapsed into two pairs of doublet of doublets (J = 45.53/10.88 Hz and 30.21/9.47 Hz). This implied that proton H_b is adjacent to H_a and the four methylene protons. On the other hand, the multiplets representing the four protons between 3.4 and 3.9 ppm remained intact upon irradiating proton H_a as shown in the bottom panel of Figure 3. This clearly confirmed that one of the vicinal protons (H_b) is adjacent to the four methylene protons.

Mechanism. Commercial Dowex 1×2 resin was prepared[1] from styrene and divinylbenzene as a 2% copolymer. The polymerization was conducted to substantial completion in the presence of benzoyl peroxide. The copolymer was then separated from the aqueous dispersion, washed with water, and dried prior to the succeeding step. The chloromethylated beads were prepared using chloromethyl methyl ether in the presence of aluminum chloride ($AlCl_3$). The reaction was controlled so that the resin product contained an average of 0.8−1.2 chloromethyl groups per aromatic nucleus.[1] The subsequent reaction with trimethylamine converted the chloromethyl moiety to the desired quaternary ammonium resin. The repeating unit, containing the quaternary ammonium salt is shown in Scheme 1.

According to the assigned chemical structure, compound **1** was determined to be a process-derived contaminant. During polymerization, a trace amount of monomeric styrene was probably entrapped in the polymer matrix and not removed by the washing and cleaning steps, and subsequent reaction with chloromethyl methyl ether resulted in the formation of **1**. Scheme 2 is the suggested mechanism that involves the reaction of chloromethyl methyl ether with styrene in the presence of anhydrous $AlCl_3$. This leads to

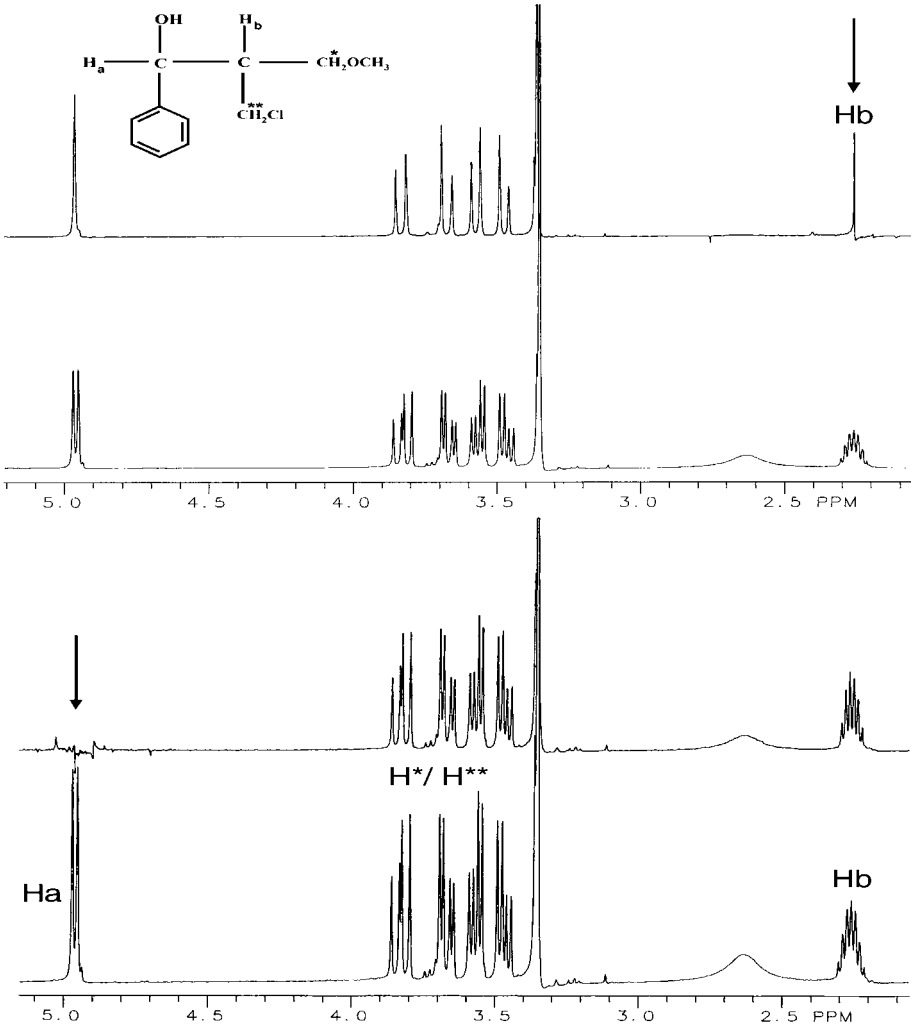
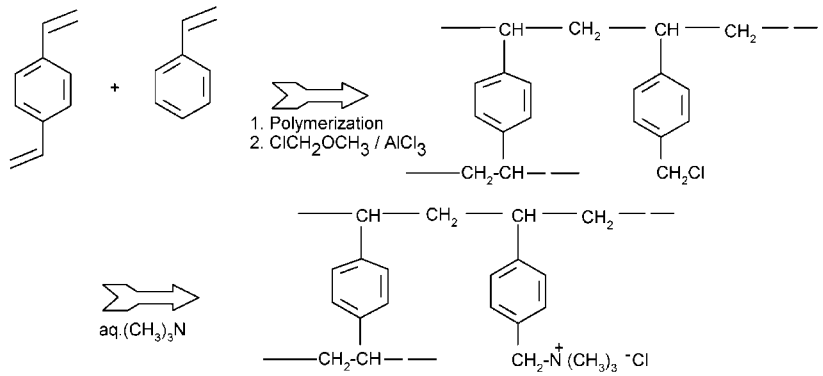


Figure 3. **Proton−proton decoupling experiments of 1: (top two panels) after irradiation of H_b and its control; (bottom two panels) after irradiation of H_a and its control.**

Scheme 1

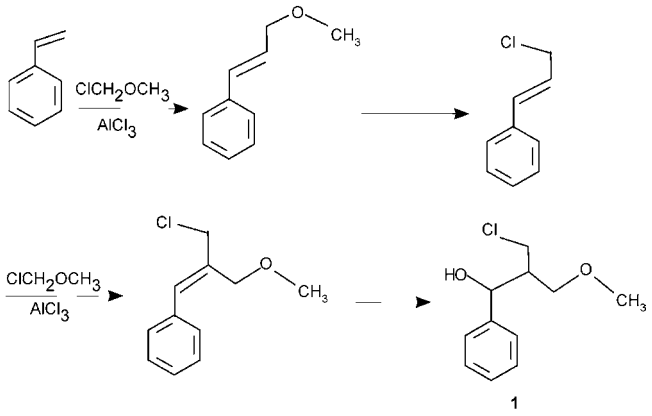


the initial modification of styrene for the formation of the monomeric species **1**.

Conclusions

Several structurally related low-molecular weight organic molecules were extracted from commercial grade Dowex 1×2 resin. The unique structural characteristics of the major organic extractable allows the unambiguous determination of its chemical structure as 2-chloromethyl-3-methoxyl-1-phenyl-1-propanol using NMR and mass spectrometry techniques. It is believed that this compound and the other small extractables were the products of styrene and chloromethyl methyl ether used in the production of Dowex 1×2 resin. They were entrapped in the polymer matrix and not removed in the subsequent washing process steps. When using this resin in chromatography, these contaminants should be removed from the resin as recommended in the manufacturer's guidelines to guarantee a food-grade quality

Scheme 2



of the ion-exchange resin. On the other hand, if the resin pre-wash is not performed, the process streams should be closely monitored; thus, high product quality can be achieved.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were performed using either a Varian XL 300 MHz or a VXR 300 MHz spectrometer. All the spectra were taken in deuterated chloroform ($CDCl_3$) and reported in parts per million (δ) downfield from tetramethylsilane. The coupling constant J was reported in Hz.

Mass spectra were obtained under low and moderate (RP = 1000) resolution analyses conditions using a VG7070EHF mass spectrometer system. Multifaceted techniques of electron impact (EI^+), chemical ionization (CI^+), gas chromatography/mass spectrometry (GC/MS), fast atom bombardment (FAB), and solid probe analyses were used in the determination of the chemical structures of these impurities. High-resolution (RP = 5000) mass spectrometry analysis was used for elemental composition confirmation using a Finnigan MAT90 magnetic sector mass spectrometer in the FAB mode only.

A Hewlett-Packard 5890 gas chromatograph equipped with a 30 m × 0.25 μ nonpolar, thin film, DB-1 capillary column was used to analyzed samples derivatized with BSTFA containing 10% trimethylchlorosilane.

RP-HPLC was performed using a Perkin-Elmer series 4 HPLC with an ISS-100 auto sampler. An analytical Vydac C18 column (218TP510, 5 μ, 4.6 mm × 250 mm) was used for the analysis of extractables. For the isolation of **1** for characterization, a semipreparative Vydac C18 column (218TP54, 5 μ, 10 mm × 250 mm) was used. The extracted solution was eluted using acetonitrile/water containing 0.2% trifluoroacetic acid and monitored with a Kratos 783 detector at A280 nm. Data were collected and analyzed with a PE-Nelson Turbochrom data system. HPLC grade TFA and acetonitrile were purchased from Baxter Scientific.

Isolation of 1. Sixty grams of Dowex 1×2 (Lot number MM910608) were placed in a beaker and stirred with 350 mL of methylene chloride for an hour at room temperature. The extracted resin was collected by filtration, and the same extraction procedure was repeated three times. The combined methylene chloride solutions were dried over magnesium sulfate and evaporated to dryness by a rotoevaporator. The major component **1**, was purified by a semipreparative RP-HPLC and analyzed by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. ^{1}H NMR ($CDCl_3$): δ 2.2−2.3 (m, 1H), 2.8 (s, 1H), 3.35 (s, 3H), 3.45 (dd, 1H), 3.55 (dd, 1H), 3.65 (dd, 1H), 3.85 (dd, 1H), 4.95 (d, 1H, J = 5.64 Hz), 7.2−7.4 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 141.86, 128.49, 127.75, 126.07, 74.13, 70.99, 59.21, 48.09, 41.97. Key MS m/z (rel intensity): 107 (100%), 214 (M^+, 2.6%), 216 (0.9%); BSTFA derivatized **1**: 179 (100%), 286 (M^+, 0.1%). High-resolution mass spectrometry under FAB/MS conditions: Calcd for $C_{11}H_{15}O_2ClLi$: 221.0921. Found: 221.0894.

Acknowledgment

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Supporting Information Available

Mass spectral fragmentations of **1**, ^{1}H NMR spectrum of the crude extract, and ^{13}C NMR spectrum of **1** including APT test. This material is available free of charge via the Internet at http://pubs.acs.org.

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