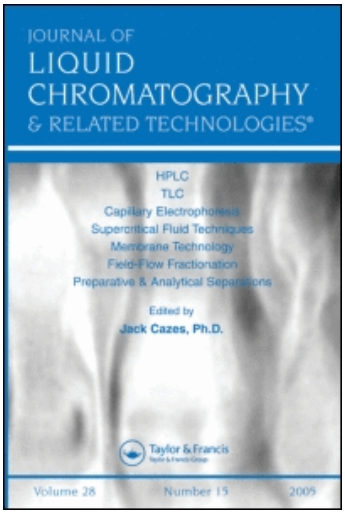

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ON THE AVAILABILITY OF 2,3,4,6-TETRA-O-ACETYL-β-D-GLUCOPYRANOSYL ISOTHIOCYANATE, A CHIRAL DERIVATIZING AGENT IN HPLC

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ABSTRACT

During the last few years, the chiral derivatizing agent 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (TAGIT) has been shown to be valuable in the separation of the enantiomers of a variety of compounds by HPLC. The sale of the reagent by its sole commercial supplier was recently discontinued, but its unacetylated analog was made available. The chemical structure of a sample of the unacetylated compound obtained from the supplier was investigated using methane and ammonia chemical ionization mass spectrometry, proton nuclear magnetic resonance, comparison of melting points, and HPLC. By all these criteria the "unacetylated" compound was shown to be, in fact, the acetylated analog, i.e., TAGIT. In view of the value of TAGIT in HPLC, its continued availability, confirmed by the supplier, is welcome.

INTRODUCTION

The high-performance-liquid-chromatographic (HPLC) resolution of enantiomers is receiving considerable attention (1-8). One approach to this problem is derivatization of the enantiomers with a chiral reagent, followed by chromatographic separation of the resulting diastereomers (2,4,6,8). Compound I, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothio-

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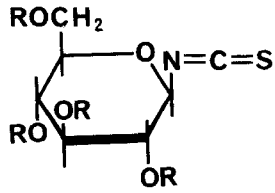
cyanate (TAGIT) was introduced recently as a chiral derivatizing reagent in HPLC (9-14). TAGIT reacts with primary and secondary amino groups rapidly and under mild conditions to give the corresponding thioureas. The reagent has been shown to be highly useful in the HPLC resolution of the enantiomers of a variety of amino acids and their esters (9,10), epinephrine and norepinephrine (11), propranolol and several related β-adrenergic antagonists (12), ephedrine, pseudoephedrine, and norephedrine (13), and several 1-phenyl-2-aminopropanes (amphetamines) (14). TAGIT has been commercially available from Polysciences Inc. (Warrington, PA), but recent attempts by us and by others (15,16) to obtain the reagent from that supplier have been unsuccessful: the sale of the compound was said to have been discontinued. Consistent with this information, the 1984-85 catalog of Polysciences listing the currently available chemicals contains no entry for TAGIT. The catalog does contain, however, an entry for β-D-glucopyranosyl isothiocyanate (catalog no. 8648, structural formula II), i.e., the unacetylated analog of TAGIT. We were interested in exploring this compound as a chiral derivatizing agent alternative to TAGIT, although the stability of II, a polyhydroxy isothiocyanate, appeared doubtful. In this communication we describe the unexpected results obtained.



	R
I	$COCH_3$
II	H

EXPERIMENTAL

Melting points were determined on Fisher-Johns melting point apparatus and are uncorrected.

TAGIT was obtained from Polysciences Inc. before the sale of the compound was discontinued. The m.p. of TAGIT was 114°-116° (lit. (9) m.p. 113-115°). Samples of purported β-D-glucopyranosyl isothiocyanate (II) were also purchased from Polysciences Inc.

Derivatization and Chromatography

The reaction of the chiral reagent with propranolol and the subsequent HPLC analysis were carried out as described previously (12).

Mass Spectrometry

Mass spectrometry was carried out using a VG Micromass 7070H (Altrincham, United Kingdom) instrument in the chemical ionization (CI) mode. Ultra pure methane (Matheson Gas, Caucaln, NJ) and ammonia were used as reagent gases. The reagent gas pressure was adjusted to maximum MH^+ (m/z 107) production for xylene which corresponds to 0.7 to 1 torr in the ion source. The electron beam was 100 eV. Samples were introduced by the solid probe with an ion source temperature of 140°.

Nuclear Magnetic Resonance

The 90-MHz proton nuclear magnetic resonance (PMR) spectra were recorded using a Varian Assoc. (Palo Alto, CA) EM 390 instrument. The compounds were dissolved in deuteriochloroform containing tetramethylsilane (TMS) as internal reference.

RESULTS

The melting point of a sample purchased as II was 113-115°, which is identical to the reported melting point of TAGIT (9). A mixed-melting-point determination with authentic TAGIT also gave 113-115°.

The ammonia CI mass spectrum of purported II is shown in Figure 1. The spectrum was identical to that of a sample of TAGIT. Similarly, the methane CI mass spectrum of the compound thought to be II (Figure 2) was identical to the spectrum of TAGIT.

The 90-MHz PMR spectrum of the two samples were also compared (spectra not shown). Again, the two spectra were identical. In particular, the acetyl methyl protons were present in both spectra, appearing as two singlets at 2.03 and 2.12 ppm downfield from TMS.

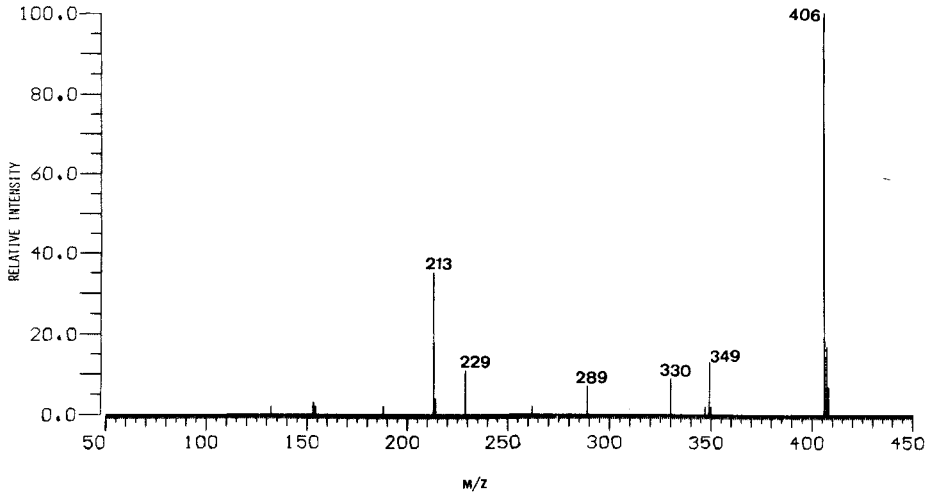


FIGURE 1 Ammonia CI mass spectrum of substance purchased as compound II.

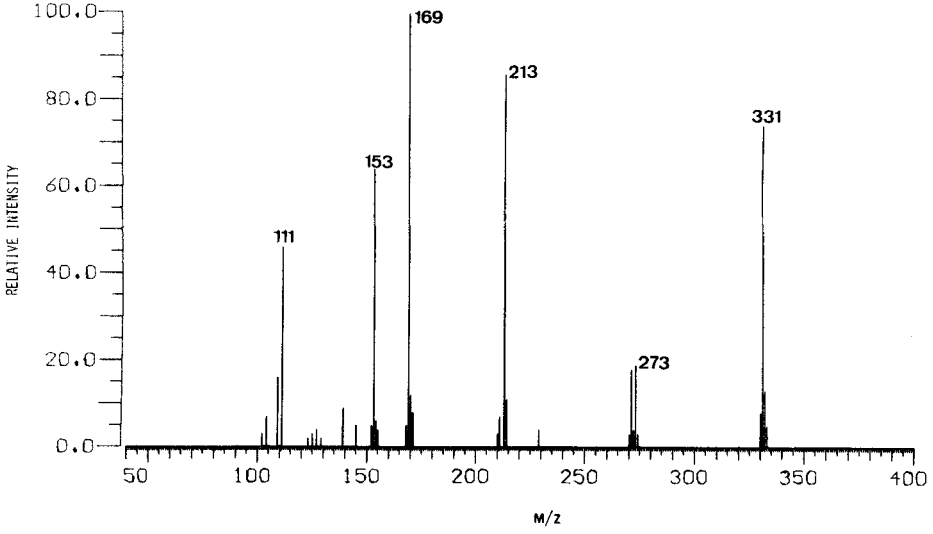


FIGURE 2 Methane CI mass spectrum of substance purchased as compound II.

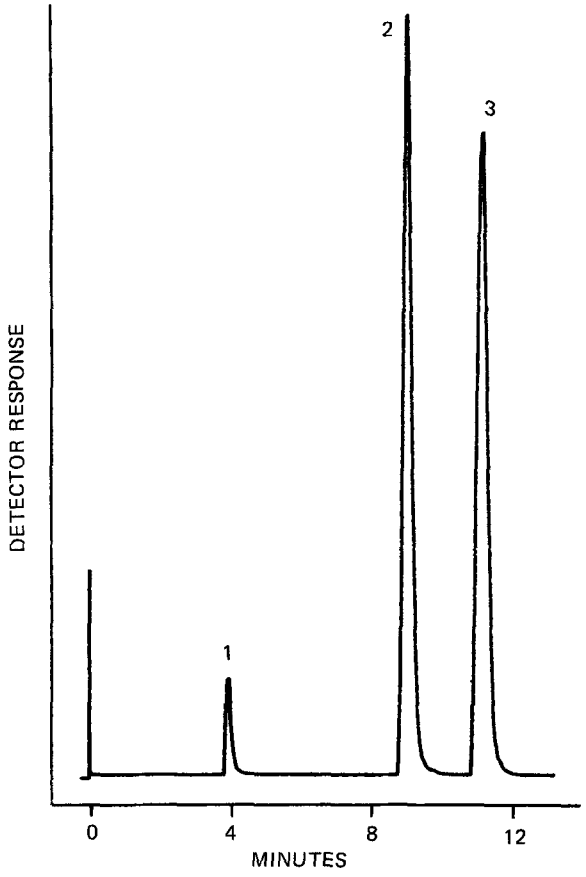


FIGURE 3 Chromatogram of racemic propranolol derivatized with purported II. Peak 1: reagent peak; peak 2: derivative of (-)-propranolol; peak 3: derivative of (+)-propranolol.

Derivatization of (±)-propranolol with purported II and HPLC analysis of the products yielded the chromatogram shown in Figure 3. Derivatization with TAGIT gave a chromatogram indistinguishable from that shown in Figure 3, and essentially identical to chromatograms obtained previously (12). When (R)-(+)-propranolol was derivatized with the reagent obtained as II, only the diastereomer with the longer retention time (peak 3 in Figure 3) was obtained. As expected, this was identical to the results obtained with TAGIT.

DISCUSSION

The substance obtained from the supplier as compound II was analyzed using several analytical techniques. By all criteria the substance was found to have, in fact, structure I and was indistinguishable from TAGIT. For example, the mass spectra of the substance (Figures 1 and 2) were consistent with structure I and were essentially identical to those of TAGIT. There was no molecular ion species observed in the methane CI mass spectrum of TAGIT (Figure 2), which was not unexpected. The major high mass ion at m/z 331 corresponds to a loss of isothiocyanate (58 daltons). The major ions arise from multiple losses of acetate and ketene. The ammonia CI mass spectrum was investigated in view of the known reactivity of TAGIT with amines to form thioureas (Figure 1). In fact the major ion observed corresponded to the ammonia attachment ion at m/z 406 (389+17 daltons). The fragmentation of this ion was greatly reduced, no doubt due to covalent bond formation.

The PMR spectra and the results of the melting-point determinations also confirmed the identity of the substance as TAGIT. Not surprisingly, when the reagent was used to derivatize propranolol, the HPLC chromatogram obtained (Figure 3) was identical to that obtained using authentic TAGIT. Furthermore, derivatization of (+)-propranolol revealed that the enantiomeric purity of the chiral derivatizing reagent was > 99.5% since no peak was detectable for the second diastereomer.

It is clear from the foregoing that the sample of compound no. 8648 in the Polysciences catalog obtained by us is in fact TAGIT. We have reported our findings to Polysciences, and the supplier agrees with our conclusion about the identity of the substance (17). Furthermore, compound II is not available from Polysciences, and it appears to us that its listing was simply an error in identifying TAGIT. We now understand that this error will be corrected and that TAGIT--correctly identified--will again be available (17). In view of the value of TAGIT as a chiral reagent in HPLC (9-14), its continued availability is indeed welcome.

ACKNOWLEDGMENTS

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