Pergamon

Tetrahedron: Asymmetry Vol. 6, No. 7, pp. 1499-1502, 1995

0957-4166/95 $9.50+0.00

0957-4166(95)00182-4

Partial Preparative Resolution of the Inhalation Anesthetic Enflurane Using Clathrate Inclusion Complexes

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Abstract: Cage-type clathrate inclusion complexes of tri-*o*-thymotide (TOT, **2**) afford partial resolution (ee = 38%) of (±)-enflurane (**1**) after a single crystallization step.

The most commonly used inhalation anesthetics today, halothane ($CF_3CHBrCl$), isoflurane ($CF_3CHClOCHF_2$), desflurane ($CF_3CHFOCHF_2$) and enflurane (**1**), all contain a stereogenic center and their preparative resolution, or asymmetric synthesis, represents both a challenging fundamental problem (how to resolve such small, apolar molecules lacking the functional groups usually used for complexation or for attachment of a chiral auxiliary) and one with wide interest ranging from the desire to study their chiroptical properties and, most importantly, to understand better their mode of anesthetic action.[1] The availability of enantiomerically pure enantiomers could also be used to establish whether these undergo different metabolism, a problem of concern in anesthesiology.

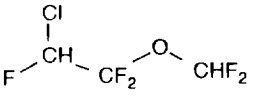
Previous attempts to prepare inclusion complexes of halothane were made using brucine,[2] α-cyclodextrin,[3] and tri-*o*-thymotide (TOT, **2**),[4] but none of these were successful. The conditions used for preparing the TOT-halothane complex afforded achiral crystals (space group *Pbca*, host: guest ratio 1:1).[5] An early report of the asymmetric synthesis of halothane involved a step known to cause racemization.[6] Recent reports describe the stereospecific stepwise syntheses of enantiomerically pure halothane, enflurane, and isoflurane from enantiomerically pure precursors, but with poor overall yields (1-2.5%).[7]

We now report that cage-type clathrate inclusion complexes of TOT afford partial resolution (ee = 38%) of **1** after a single crystallization step; such enclathration may thus provide a mode for obtaining sufficient quantities of the two enantiomers to carry out both chiroptical and biological studies (see discussion of approaches to increase the ee below).

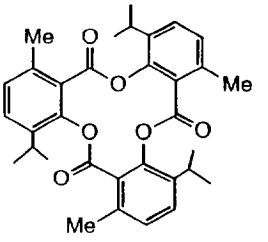
In solution, TOT is a rapidly interconverting racemic mixture of stereogenic structures; on crystallization with suitable guest substances, well-developed chiral clathrate crystals are formed ("spontaneous resolution").[11] Since the TOT molecules are all homochiral in each single crystal (but the crystals are not hemihedral and cannot be visually distinguished as right- or left-handed), the enclathrated guest should be enantiomerically enriched. If dissolved at ca. 0°C, the TOT optical rotation, $[\alpha]_D$~70°C, can be measured before it fully racemizes and then, after the TOT rotation reaches zero, the rotation of guest

can be measured if its concentration and [α] are sufficiently high. Direct GC, HPLC, or NMR methods are best used to establish enantiomeric ratios.

Crystallization of TOT from solutions of **1** in 2,2,4-trimethylpentane (TMP) afforded chiral cage-type clathrate crystals (space group $P3_121$, host:guest ratio 2:1). Gas chromatographic analytical resolution of halothane, isoflurane, and **1** had earlier been reported using a cyclodextrin derivative.[8] Using the polymeric chiral phase Chirasil-Dex coated on a capillary GC column,[9] enflurane was quantitatively separated at low temperature (Figure 1a). Using this column, it could be shown that TOT-enflurane single crystals contained 38% ee of the guest. As expected (and this represents an important control, ruling out artifactual ee observations), for some clathrate crystals the *R*-(-)-enantiomer that eluted first predominated, while in others the *S*-(+)-enantiomer that eluted last predominated. The ee was identical in replicate crystals of both kinds (See Figure 1b,c).



Enflurane (**1**)



Tri-*o*-Thymotide (TOT, **2**)

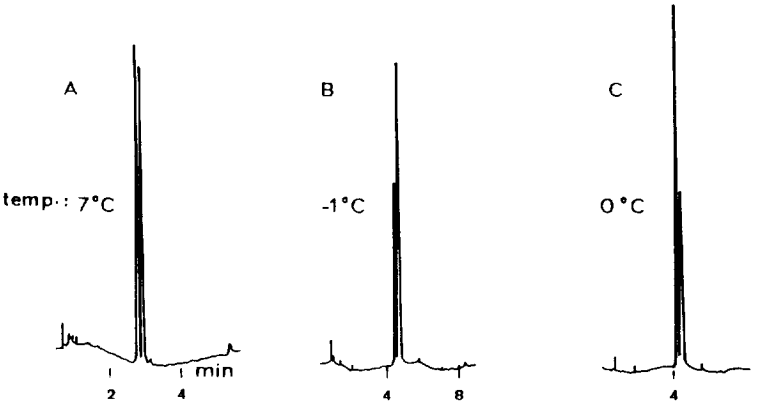


Figure 1. Resolution of the inhalation anesthetic enflurane. Headspace analysis either of vapor above the liquid or after heating a single clathrate crystal > 140°C. Column: 25 m x 0.25 mm i.d. coated with Chirasil-Dex,[9] d_f = 0.25 μm. Temperatures (°C) given are of the column during the analysis. (a) Racemic material; (b) a representative crystal containing 38% ee of *R*-(-)-enflurane (c) a representative crystal containing 38% ee of *S*-(+)-enflurane.

Seeding of saturated solutions of TOT in a guest with selected homochiral single crystals produces large polycrystalline samples, which can afford relatively large amounts of enantiomerically enriched guest.[10] Furthermore, repeated crystallization of TOT clathrate crystals leads to an additive, enhanced ee.[11] Finally, since the absolute configuration of TOT is known,[12] the X-ray structure of a clathrate crystal could provide the absolute configuration of an inhalation anesthetic guest molecule. Related clathrate hosts[13] also provide chiral inclusion compounds with inhalation anesthetics.[14] These approaches are now being studied in order to obtain large amounts of enantiomerically pure, or highly enriched, material for further studies.

Experimental Section: TOT was synthesized from *o*-thymotic acid by treatment with $POCl_3$ and purified by crystallization from hot ethanol. Solvent-free material was obtained by warming the crystalline TOT-ethanol complex to 130°C at 0.2 mm/Hg for 24 h and recrystallization from methanol.[15] The TOT-enflurane clathrates were prepared by dissolving solvent-free TOT (0.2 g) in boiling enflurane (1.0 ml), then adding TMP (9.0 ml). The resulting solution was filtered and cooled slowly to 0°C (24 h). The crystals were filtered and washed with cold methanol (1 ml), giving 0.08 g of large, colorless cubic crystals, mp = 165-170 °C, with a TOT:enflurane ratio of 2:1, as indicated by ^{1}H-NMR and microanalysis. The function of the TMP is apparently twofold: it favours cage-type clathrate nucleation and lowers the density of the solution, preventing the growing crystal from floating on the top of the solution. The resulting TOT-enflurane clathrate inclusion complex contains no TMP.

Acknowledgements: This work was supported by the U.S.-Israel Binational Science Foundation (BSF) Grant No. 84-00162, Jerusalem (Israel), Deutsche Forschungs-Gemeinschaft, and Fonds der Chemischen Industrie.

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(*Received in UK* 18 *April* 1995; *accepted* 13 *June* 1995)