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Gas chromatographic assay for 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane

STEFANO A. POGÁNY* and RODNEY A. DEEKEN
INTERx Research Corp., Merck Sharp & Dohme Research Laboratories, 2201 West 21st Street, Lawrence, KS 66047 (U.S.A.)
and
GAYLEN M. ZENTNER
INTERx Research Corp., Merck Sharp & Dohme Research Laboratories, 2201 West 21st Street, Lawrence, KS 66047 (U.S.A.), and Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045 (U.S.A.)
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SUMMARY

A gas chromatographic method was developed, based on a reference standard, for analysis of the reactive diketene acetal 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane (DETOSU). The method is based on the conversion of DETOSU to 3,9-diethyl-3,9-dimethoxy-2,4,8,10-tetraoxaspiro[5.5]undecane, a stable *ortho* ester.

INTRODUCTION

Ketene acetals react with alcohols to generate ortho esters[1]. 3,9-Diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane (DETOSU)[2] is a bifunctional ketene acetal used in reactions with diols to synthesize polymeric ortho esters which are hydrolytically unstable and have applications in the area of controlled drug release[3]. DETOSU is a reactive electrophile. Some of its known reactions[1,4,5] are hydrolysis, acid catalyzed self-polymerization, base catalyzed double bond migration, partial hydrolysis followed by intramolecular cyclization and nucleophilic attack by hydroxylic compounds such as alcohols and carboxylic acids. The reaction of DETOSU with polyols to produce polymeric *ortho* esters [poly(ortho ester)s] is a step-reaction (condensation polymerization) in which accurate stoichiometry and high purity monomeric starting materials are critical to obtain high degrees of polymerization[6,7]. It is, therefore, necessary to know accurately the purity of the polyols and the DETOSU. The determination of DETOSU purity presents a special problem because of its highly reactive nature and attendant lack of a reference standard. In the past, DETOSU purity was estimated by an area% gas chromatographic (GC) assay[8,9] that was not based on a reference standard. Non-volatile contaminants (*e.g.* oligomers) would not be detected and inaccurate purity results were possible. This paper presents a

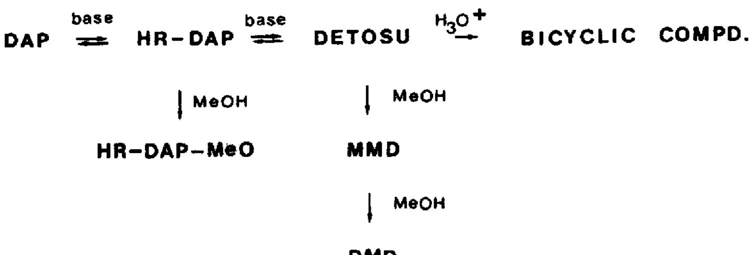

Fig. 1. Synthesis of DETOSU. Me = Methyl.

quantitative GC assay method for DETOSU purity based on the conversion of DETOSU to a stable derivative, dimethoxyDETOSU, that may be isolated for use as a reference standard.

To understand the origin of the peaks in the chromatograms a brief review of DETOSU's synthesis is helpful (Fig. 1). DETOSU is obtained from diallylidene pentaerythritol (DAP) by base catalyzed isomerization of the two double bonds of the diallyl acetal. The isomerization reaction does not lead quantitatively to DETOSU; instead an equilibrium mixture is established the composition of which is unaffected by longer reaction times and/or higher temperatures. The three main constituents of the final reaction mixture are DETOSU, the half-rearranged material (HR-DAP) in which only one double bond is shifted and a bicyclic ortho ester that presumably originates from the reaction of DETOSU with one molecule of water producing a hydroxymethyl group which adds to the residual ketene acetal moiety within the same DETOSU molecule. The reaction of HR-DAP with one molecule of methanol produces HR-DAP-CH_3O. The reaction of DETOSU with one or two molecules of methanol generates monomethoxyDETOSU (MMD) or dimethoxyDETOSU (DMD), respectively.

MATERIALS AND METHODS

The diketene acetal DETOSU was provided by Merck. Methanol (Burdick & Jackson high-purity brand) and methyl myristate (Aldrich, 99% purity) were used as received. Helium, nitrogen and hydrogen (ultra-high-purity grades; Linde Specialty Gases) and compressed air (hydrocarbon-free grade; Linde Specialty Gases) were used as received.

3,9-Diethyl-3,9-dimethoxy-2,4,8,10-tetraoxaspiro[5.5]undecane (DMD) was synthesized by heating DETOSU in methanol at reflux for 1 h, purifying by vacuum distillation (0.1 mmHg, 120°C) and recrystallization [0°C, from light petroleum (b.p. 30–60°C)]. Fourier transform infrared (FT-IR) (Nicolet 5DX FT-IR; KBr pellet), proton NMR (Bruker ACE-200; tetramethylsilane internal standard) and elemental (Schwarzkopf Microanalytical Labs, N.Y., U.S.A.) analyses were performed to verify the molecular structure of DMD. The melting point and purity of DMD were determined by differential scanning calorimetry (DSC) (Perkin-Elmer DSC-4). The DSC celll was calibrated using an indium standard. Dry nitrogen was used to purge the cell for all measurements. Sealed DSC pans with sample sizes ranging from 2 to 5 mg were used in all measurements. To insure good thermal contact between sample and pan,

each sample was briefly heated to 70°C (melted) and then rapidly cooled to −25°C and allowed to stand for 1 h to permit complete recrystallization. The temperature was then raised (100°C/min) to 35°C and the purity runs performed at 2°C/min over the temperature range of 35 to 70°C.

A gas chromatograph (Hewlett-Packard 5840A) equipped with a flame ionization detector, split/splitless capillary inlet port and a fused-silica capillary column (J & W Scientific DB-1 Megabore™, 15 m × 0.530 mm I.D.) with a cross-linked 100% methyl silicone stationary phase (1.5-μm film thickness) was used. The carrier gas was helium (flow-rate: 10 ml/min) and the detector was supplied with the appropriate flows of hydrogen, air and nitrogen as a make-up gas. The temperatures of the inlet port and detector were 250°C. The column temperature was 150°C isothermal. The volume injected was approximately 2 μl with a split ratio of 1:10.

The GC–mass spectrometric (MS) experiments were performed with a gas chromatograph (Hewlett-Packard 5890) equipped with a mass selective detector (Hewlett-Packard 5970B). The mass spectrometer source was operated at 70 eV with electron impact (EI) ionization. A fused-silica capillary column (HP-1 cross-linked 100% methyl silicone, film thickness 0.33 μm, 12 m × 0.20 mm I.D.) with a split/splitless capillary inlet port was used. The carrier gas was helium (flow-rate: 1 ml/min). The injection volume was approximately 0.5 μl with a split ratio of 1:10. Other chromatographic conditions were the same as described previously.

An internal standard solution (ISS) was prepared by dissolving 4.1 g of methyl myristate in 1000 ml of methanol. A reference standard stock solution (PSS) was prepared by dissolving 0.5184 g of DMD in 25 ml methanol. Three standards were prepared by transferring via pipette 1, 2 and 3 ml, respectively, of the PSS into 25-ml volumetric flasks containing 10 ml of ISS, then filling to volume with methanol.

DETOSU samples were prepared by accurately weighing approximately 50 mg into 25-ml volumetric flasks containing 10 ml methanol then heating at 60°C for 5 h, routinely, with longer equilibration times also investigated. After cooling to room temperature, 10 ml of ISS were added and the flasks were filled to volume with methanol.

RESULTS

Analysis of the synthetic DMD by several methods confirmed its identity. The proton NMR spectra (taken in [$^2H_6$]benzene and in [^2H]chloroform) were consistent with its structure. The FT-IR spectrum revealed bands at 2978, 2969, 2948, 2913, 2879, 1465, 1458, 1357, 1204, 990, 956, 943 and 926 cm^{-1}. Elemental analysis of DMD ($C_{13}H_{24}O_6$, mol.wt. 276.33) yielded values for carbon and hydrogen of 56.59% and 8.78%, respectively (theoretical values: 56.51% and 8.75%). The melting point of DMD was 51.2°C and the purity (DSC) was 98.2 ± 0.2 mole% ($n = 6$). DMD (solid) was stable for > 1 year as indicated by DSC analysis when stored at −25°C in glass bottles.

Injection of authentic samples and GC–MS analysis were used to assign various peaks in the DETOSU sample chromatograms. Authentic samples of DAP, DETOSU and DMD were available and exhibited elution times of 2.22, 3.26 and 6.87 min, respectively. The identities of the other peaks were determined by GC–MS. The peak at 2.72 min had the same molecular ion (m/z = 212) as DAP. This was consistent with

the DAP isomer, HR-DAP, since both compounds have the same molecular weight. The peak at 3.17 min (an impurity in the internal standard) was identified as methyl decanoate by a probability-based library match of the GC–MS data (UNIX Chemstation™ software; Hewlett-Packard). The peak at 3.53 min was previously identified[5] as the bicyclic ortho ester 1-propanoyloxymethyl-4-ethyl-2,6,7-trioxabicyclo[2.2.2]octane and exhibited a weak molecular ion at m/z = 230 and a prominent fragment ion from ester cleavage at m/z = 57 due to $CH_3CH_2CO^+$. Although the peak eluting at 3.84 min was not always discernible due to its low concentration, this peak may be assigned with some confidence to HR-DAP-CH_3O. The addition of methanol to impure DETOSU containing HR-DAP resulted in the disappearance of the HR-DAP peak (2.72 min) and the corresponding appearance of the peak at 3.84 min. The peak at 4.84 min was assigned to MMD. As the reaction of DETOSU with methanol proceeded, this peak first increased then decreased in size, as expected in an A → B → C reaction.

The peak at 4.84 min never disappeared completely and was directly proportional to the DMD concentration in both standards and samples as illustrated in Fig. 2. Variation of the inlet temperature from 150 to 300°C in an attempt to alter the amount of MMD formed during a possible *in situ* breakdown process, resulted in no change in the MMD peak size. Lowering the column temperature resulted in peak tailing, while increasing the column temperature above 150°C resulted in loss of resolution. The mass spectrum associated with this peak showed that it consisted of a single component and exhibited a molecular ion at m/z = 244 (molecular weight of MMD) and an $[M+1]^+$ ion at m/z 213 (molecular weight of DETOSU) attributable to loss of one molecule of methanol from MMD. The remaining fragmentation pattern could be recognized as that of DETOSU. The peak at 7.71 min was methyl myristate, the internal standard.

The structures of the various species and the respective GC retention times are summarized in Table I.

A plot of the DMD–internal standard peak-area ratio *versus* the DMD concentration was linear (r^2 = 0.99) over the concentration range of 0.9 to 4.6 mg/ml. Periodic GC analysis of standard solutions (stored at −25°C) indicated stability for at least two months; linear least squares regression slopes and intercepts of cali-

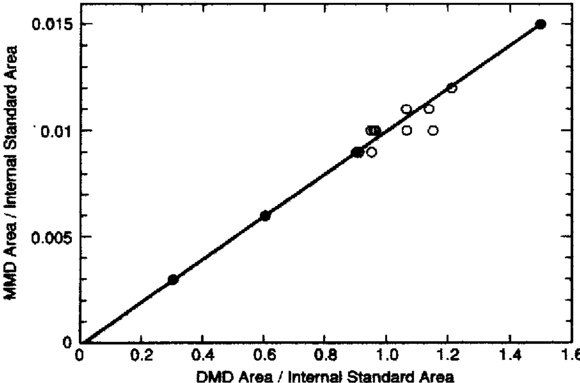

Fig. 2. MMD proportionality to DMD (r^2 = 0.9999). ● = standards; ○ = samples.

TABLE I

IDENTIFIED COMPOUNDS AND CORRESPONDING RETENTION TIMES

Compounds		*Retention time (min)*	
1		DAP	2.22
2		HR-DAP	2.72
3		DETOSU	3.26
4		HR-DAP-CH_3O	3.88
5		Bicyclic compound	3.53
6		MMD	4.84
7		DMD	6.87
8		Methyl myristate	7.71

TABLE II

STABILITY OF STANDARD SOLUTION CALIBRATION CURVES

Time (months)	*Slope*	*Intercept*
0	0.2908	0.0042
	0.2915	0.0037
	0.2967	0.0040
Average	0.2930 ± 0.0032	0.0040 ± 0.0003
2	0.2975	0.0047
	0.2959	0.0072
	0.2961	0.0086
Average	0.2965 ± 0.0009	0.0068 ± 0.0020

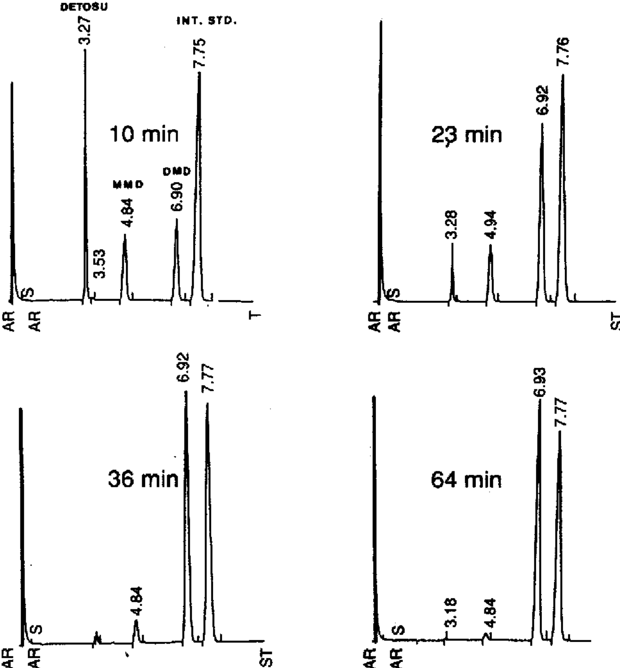

Fig. 3. Chromatograms illustrating the conversion of DETOSU to DMD when heating at 60°C for 10, 23, 36 and 64 min.

bration curves generated from the same standard solutions at time-zero and at time-two months were essentially the same (Table II).

The intraday precision of the assay, defined as the % coefficient of variation (C.V.) was 0.33%. This number, based on ten replicate injections of the same sample, was calculated by dividing the value of the standard deviation by the mean value (n = 10) of the DMD–Internal Standard peak-area ratio and expressing the quotient as a percentage.

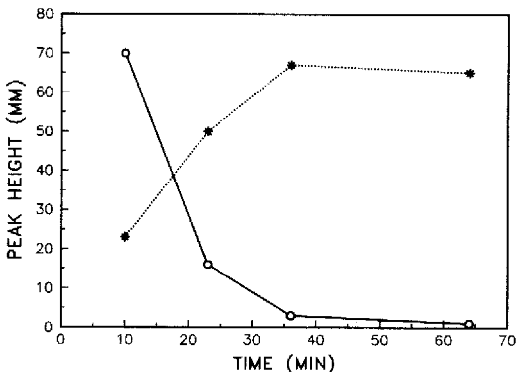

Fig. 4. Kinetics of DMD (*) formation from DETOSU (o).

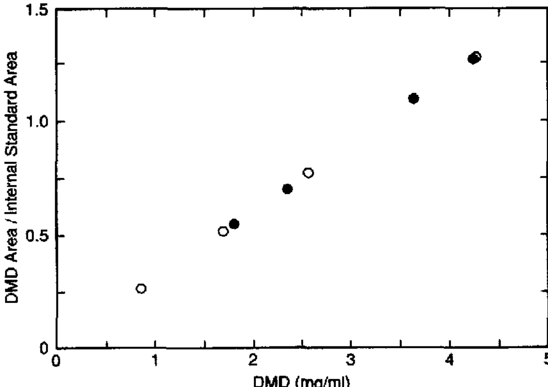

Fig. 5. Extent of conversion of DETOSU to DMD. ● = DETOSU converted to DMD; ○ = DMD standards.

The formation of DMD from DETOSU was followed as a function of time by GC (Figs. 3 and 4). Complete reaction had occured within 1 h as evidenced by the disappearance of the DETOSU peak and stable values for the DMD peak. Longer reaction times (2, 6, 18 and 48 h) at 60°C did not result in further increase of the DMD peak area.

To validate the accuracy of the assay, the extent of conversion of DETOSU to DMD was examined. The data are summarized in Fig. 5. The standard curve was obtained by injection of four DMD standards. This was compared to the sample curve obtained from four DETOSU samples that had been derivatized with methanol as described previously. The following two assumptions were made: (1) the DETOSU was 100% pure and (2) the conversion of DETOSU to DMD was 100%. The sample curve is the plot of the GC area response *versus* the theoretical concentration of DMD that would be obtained if 100% pure DETOSU converted quantitatively to DMD. The standard and sample curves have similar slopes and intercepts (slopes: 0.2982, 0.2961; intercepts: 0.0029, 0.0086) indicating a quantitative conversion of DETOSU to DMD. These data were analyzed by Student's t-test method[10] and no significant difference ($p > 0.5$) was found between the slopes of the two curves.

DISCUSSION

The results of this study indicate that the conversion of DETOSU to DMD is quantitative despite the persistence in both the sample and standard chromatogram of a minor peak at 4.84 min identified as MMD by GC–MS. The reason why this residual amount of MMD should not convert to the dimethoxy derivative under the sample preparation conditions of the assay could not be determined. It is proposed that the small peak persisting at 4.84 min is due to an equilibrium between MMD and DMD established inside the injector or column of the gas chromatograph. This hypothesis was corroborated by the fact that the amount of MMD was directly proportional to the amount of DMD injected and that repeated recrystallizations of DMD standards were not effective in reducing the MMD residue peak. The quantitative conversion of DETOSU to DMD, a stable material that can be isolated in high purity

and stored for long periods of time without degradation, can be used in a quantitative assay of DETOSU based on a reference standard.

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