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Note

Preparation and gas–liquid chromatography of trimethylsilyl derivatives of dammarane-type triterpene triols and tetrols

T. V. POKUSHALOVA*, L. I. GLEBKO, N. D. POKHILO and N. I. UVAROVA
Pacific Institute of Bioorganic Chemistry, Far East Science Centre, USSR Academy of Sciences, Vladivostok 690022 (U.S.S.R.)
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Dammarane-type triterpene alcohols isolated from birch leaves represent interesting starting materials for the preparation of glycosides, such as synthetic analogues of panaxosides[1–3]. Recently, new triterpene alcohols belonging to this series have been isolated from birch trees widespread in the Soviet Union[4,5], and gas–liquid chromatography (GLC) has been used for evaluating the triterpene composition of the crude extracts.

The preparation and chromatographic properties of the trimethylsilyl (TMS) ethers of dammarane-related alcohols having a tetrahydrofuran ring in their side-chain have been reported earlier[6]. In this note we describe the TMS derivatives of related compounds with acyclic side-chains.

EXPERIMENTAL

GLC separations were carried out on a Tsvet-104 instrument equipped with a flame ionization detector and glass columns (3 m × 3 mm). The columns were packed with either 1.5% OV-1 (working temperature, 245°C) or 1.5% OV-17 (260°C) on Chromaton N-super (0.250–0.315 mm), and deactivated by several 10-μl injections of hexamethyldisilazane. The carrier gas (helium) flow-rate was 65 ml/min, and the samples (2–4 μl) were injected directly on the columns. The mass spectra were recorded at 70 eV on a GLC-MS LKB-9000 instrument fitted with a SE-30 column.

Preparation of N-trimethylsilylimidazole

TMSI was prepared from imidazole and hexamethyldisilazane according to a literature procedure[9].

Preparation of TMS ethers

Method A. To a sample (0.1–0.2 mg) of the alcohol in a screw cap test-tube, dried pyridine (30 μl), TMSI (30 μl) and TMCS (10 μl) were successively added. After 1.5 h at room temperature, the mixture (4–5 μl) was directly injected on the GLC column.

Method B. To a sample (0.2–0.3 mg) of the alcohol in a screw cap test-tube (lubricated with silicone), dried pyridine (30 μl), TMSI (60 μl) and TMCS (30 μl)

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were successively added. After 6 h at 100–110°C, the mixture (4–5 μl) was directly injected on the GLC column.

Preparation of dammara-3α,12β,20(S)-triol

Alcohol I (30 mg) was dissolved in pure acetic acid (5 ml) and stirred for 1 h 40 min at room temperature under hydrogen in the presence of Pt (Adams' catalyst, 3.0 mg). The catalyst was filtered off, the solution was diluted in water (15 ml) and the product extracted with diethyl ether (65 ml).

RESULTS AND DISCUSSION

The dammarane-type alcohols I–VI (Fig. 1, Table I) differ in the number (3 or 4), location [at C(3), C(12), C(17), C(20), C(24)] and configuration of the OH groups, whereas their side-chains are unsaturated either at C(24) or C(25). The general structure of these polyalcohols with acyclic, unsaturated side-chains makes them relatively unstable under GLC conditions, as demonstrated by the appearance of asymmetric, broad and multiple peaks on their chromatograms. That such compounds had to be derivawtized prior to chromatography was obvious and for this purpose we planned to use the TMS ethers, as previously described[6].

The silylation of the tertiary OH groups at C(17) and C(20) in these compounds required the use of a powerful reagent, *i.e.*, N-trimethylsilylimidazole (TMSI). As the more reactive, secondary OH groups at C(3), C(12) and C(24) were instantly silylated in the presence of this reagent, the selection of conditions for the silylation of alcohols I–VI was chiefly based on the yield of the ethers of the less reactive HO-C(17) and HO-C(20) groups.

A complete silylation (80–90% isolated yield) of compounds IV and V was achieved in pyridine-TMSI (1:1, v/v), after 3 h at room temperature, but the HO-C(20) group in I–III and VI reacted only partially under these conditions. Thus, in the case of compounds I and II, only 15% of the HOC(20) group was etherified, while this group remained intact in dammar-24-ene-3α,12β,17α,20(S).tetrol (III). Clearly, the silylation of the HO-C(20) group appears to be considerably more difficult when the starting compound contains either a β-OH group at C(12) or an α-OH group at C(17). When these groups are simultaneously present, nearly complete inactivation of the HO-C(20) group results. The reactivity of the HO-C(20) group in the compounds investigated varies in the order: IV, V > II > I > VI > III.

An increase of the reaction time to 24 h was not sufficient to ensure complete silylation of the HO-C(20) group, as observed in the case of dammar-24-ene-3α,12β,20(S)-triol (I) (yield 50%). After 4 h of reaction at 100°C, a complete silylation

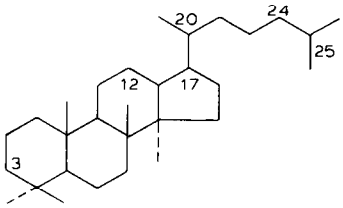

Fig. 1. Dammarane numbering scheme.

TABLE I

RETENTION TIMES (RRT) RELATIVE TO CHOLESTANE OF TMS ETHERS OF DAMMARANE-TYPE TRITERPENE ALCOHOLS

Numbering according to Fig. 1.

Compounds	*RRT*	
	OV-1	*OV-17*
I. Dammar-24-ene-3α,12β,20(S)-triol		
di-TMS ether	3.56	3.82
tri-TMS ether	4.31	3.17
II. Dammar-24-ene-3α,17α,20(S)-triol		
mono-TMS ether	5.21	6.66
di-TMS ether	5.65	5.11
tri-TMS ether	6.18	4.56
III. Dammar-24-ene-3α,12β,17α,20(S)-tetrol		
di-TMS ether	4.06	4.30
tri-TMS ether	5.75	4.61
tetra-TMS ether	6.87	4.53
IV. Dammar-25-ene-3α,20(S).24(S)-triol		
di-TMS ether	5.63	5.38
tri-TMS ether	5.46	3.68
V. Dammar-25-ene-3β,20(S),24(S)-triol		
di-TMS ether	7.52	7.20
tri-TMS ether	7.37	4.98
VI. Dammar-25-ene-3α,12β,20(S),24(S)-tetrol		
tri-TMS ether	4.79	4.14
tetra-TMS ether	5.87	3.70
Cholestane	1	1

of compound I could be obtained, but only 7% of the HO-C(20) group in III was etherified under these conditions. Fortunately, addition of trimethylchlorosilane (TMCS) to the reaction mixture dramatically enhanced the etherification rate and allowed quantitative derivatization of the HO-C(20) group, except in the case of compound III (85%). By using these conditions, *i.e.*, pyridine–TMSI–TMCS (3:3:1), room temperature, 1.5 h, even the α-HO-C(17) group in compound II was etherified to some extent (2.5%) and, after 24 h, the corresponding tri-TMS ether was produced in a 40–50% yield. However, the α-HO-C(17) group in III was not silylated at all under these conditions.

The reaction of compound II at 100–110°C with pyridine–TMSI–TMCS (1:2:1) yielded 80% of the tri-TMS derivative after 3.5 h, or 95–97% after 6 h. A similar treatment of III proved relatively disappointing in that the α-HO-C(17) group was etherified little (7%). Any further extension of the reaction time would have made the procedure useless in practice.

The steric hindrance brought about by the TMS-OC(12) group may explain the low reactivity of the HO-C(17) group in compound III, as compared to that in II. Related silylation problems presented by hindered OH groups have been reported

for compounds such as ecdisones, 20;hydroxyecdisones[7] and certain steroids[8].

In summary, satisfactory silylation procedures for alcohols I–VI appear to be those that leave the α-HO-C(17) group unchanged, either in general (Experimental, Method A) or only in the particular case of dammar-24-ene-3α,12β,17α,20(S)-tetrol (III) (Method B).

In GLC, the TMS ethers of alcohols I–VI give sharp peaks with the relative retention times given in Table I. Sometimes, however, an additional peak appears at a shorter retention time than the major peak, representing 4–8% of the area of the latter. In the case of compound I, this extra peak was identified by GLC–mass spectrometry (MS) as the TMS derivative of the di-unsaturated diol produced by thermal elimination of the TMS-OC(20) group. This is confirmed by the appearance in the mass spectrum of a molecular ion at $m/z = 586$ accompanied by fragment ions at $m/z = 571$ (M − 15), 496 (M − 90), and 297 [M − (109 + 2 × 90), where $m/z = 109$ corresponds to the di-unsaturated side-chain].

The double bond present at C(24) or C(25) exerts no obvious influence on the elimination of the TMS-OC(20) group. Indeed, the tri-TMS derivative of dammara-3α,12β,20(S)-triol, obtained by catalytic hydrogenation of compound I, also gave a "dehydration" peak in GLC.

Although all our observations and experiments led to the conclusion that these elimination reactions take place exclusively on the GLC columns, we failed to identify the factors that control their occurrence. These side-reactions were observed to be quite random in numerous repetitive tests involving the same samples, chromatographed on the same columns (of various types) and prepared according to either Method A or B. Several factors are likely to be responsible for the occurrence or non-occurrence of such elimination reactions. Interestingly, the stability of the tri-TMS ether of compound I under GLC conditions is much lower than that of the corresponding derivative of III. This suggests that, in the latter, the TMS-OC(20) group is stabilized by the vicinity of both the TMS-OC(12) and α-HO-C(17) groups. No elimination reactions or other transformations were noted during the preparation of the TMS ethers of alcohols I–VI in test-tubes, even when they were kept for several days in the reaction mixture before isolation by extraction with *n*-hexane in the presence of water.

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