Gas Chromatographic Separation of Tocotrienols: Validation of Predicted Retention Data

A RECENT PUBLICATION from this laboratory (1) described the identification and quantitative estimation of the tocopherols by gas-liquid chromatographic (GLC) separation of their trimethylsilyl (TMS) ethers. Standards were available for only two of the seven tocotrienols: 5,8-dimethyltocotrienol (5,8-T-3) and 5,7,8-trimethyltocotrienol (5,7,8-T-3). Retention data for the remaining five tocotrienols were predicted on the basis of the Kováts Retention Indices of these two tocotrienols and of the tocols. Two additional tocotrienols, 8-methyltocotrienol (8-T-3) and 7,8-dimethyltocotrienol (7,8-T-3), as well as 5,7,8-T-3, are found in latex, as reported by Dunphy et al. (2). This communication describes the chromatographic behavior of the tocopherols from a comparable sample of latex, for the purpose of validating our previous predictions regarding the retentions of the tocotrienols.

The lipid from 5 g of latex (Uniroyal "Lotol," 62.4% solids, NH_3 stabilized, from the Chemical Division of Uniroyal, Inc.) was extracted with 100 ml of chloroform/methanol 2/1. Water (20 ml) was added and the chloroform (lower) phase separated, dried, and evaporated under vacuum. The residue was dissolved in ethanol and saponified under nitrogen, with pyrogallol added as an antioxidant. The unsaponifiable residue was extracted with petroleum ether, washed with water, freed of solvent in a stream of nitrogen, and then dissolved in 0.2 ml of benzene. A 10-μliter sample of this solution was evaporated to dryness in a stream of nitrogen and the residue dissolved in 0.1 ml of a mixture of hexamethyldisilazane, trimethylchlorosilane, and dry pyridine (9:6:10) to form the TMS ethers. The solution was allowed to stand for at least 15 min and then chromatographed without further treatment on both SE-30 and Apiezon L columns, using octacosane as a reference compound. The pertinent data are given in Table I. Retention ratios ($C_{28}H_{58}$ = 1.00) for the peaks assigned to 8-T-3 and 7,8-T-3 agreed with the predicted values within the expected experimental variation.

Although the chromatographic evidence indicated that these peaks were correctly identified, some further proof seemed desirable in view of the complexity of natural products such as latex. The unsaponifiable lipid from 2.5 g of latex was derivatized with 0.2 ml of the silylating mixture. The TMS ethers were chromatographed at 250C on a glass preparative column, ¼-in. by 17 ft, packed with 1% SE-30 on Gas-Chrom Q (100/120). The three peaks identified as tocotrienols were collected as separate fractions from 25-μliter samples by passing the column effluent through 1.5–3.0-ft lengths of 16-gauge Teflon tubing, from which they were recovered by washing with diethyl ether. After the excess solvent was removed, the collected fractions were chromatographed on analytical columns to assure the survival of the intact TMS ether. For all samples the positions of the major peaks were unchanged. The individual fractions were then hydrogenated by the method of Farquhar et al. (3). Hydrogenolysis also took place, yielding free tocols, which were rederivatized to form the tocol TMS ethers. The retention ratios and Kováts Retention Indices of the TMS ether fractions before and after hydrogenation are shown in Table II. The 5,7,8-T-3 derivative was also collected and hydrogenated as a check on the procedure. Each tocotrienol was converted to the analogous saturated tocol. These transformations confirm the validity both of the peak identifications and of the predicted retention data.

TABLE I

Retention Ratios of TMS Ethers of Tocotrienols from Latex (Predicted Values Given in Parentheses)

Column	Peak No.	Identity	Area per cent	Retention ratio (RR) $C_{28}H_{58}$ = 1.00
SE-30	1	8-Methyltocotrienol	2.7	1.69 (1.71)
	2	7,8-Dimethyltocotrienol	85.7	2.23 (2.26)
	3	5,7,8-Trimethyltocotrienol	11.6	3.17
Ap. L	1	8-Methyltocotrienol	2.4	1.39 (1.38)
	2	7,8-Dimethyltocotrienol	87.3	1.94 (1.90)
	3	5,7,8-Trimethyltocotrienol	10.3	3.16

TABLE II

Retention Data of Unsaturated Tocotrienol TMS Ether Fractions Before and After Hydrogenation

Fraction No.	RR $C_{28}H_{58}$ = 1.00		Kováts Retention Index		Identity
	Ap. L	SE-30	Ap. L	SE-30	
1	1.39	1.69	2,896	2,980	8-Methyltocotrienol
1a[a]	1.05	1.29	2,814	2,889	8-Methyltocol
2	1.94	2.23	2,992	3,081	7,8-Dimethyltocotrienol
2a[a]	1.46	1.70	2,910	2,982	7,8-Dimethyltocol
3	3.16	3.17	3,134	3,207	5,7,8-Trimethyltocotrienol
3a[a]	2.35	2.44	3,048	3,113	5,7,8-Trimethyltocol

[a] Hydrogenated.

HAL T. SLOVER
ROBERT J. VALIS
JOANNA LEHMANN
Human Nutrition Research Division
USDA, ARS
Beltsville, Maryland 20705

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