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GAS–LIQUID CHROMATOGRAPHY OF RESIN ACID ESTERS

D. F. ZINKEL and C. C. ENGLER

*Forest Products Laboratory**, *Forest Service U.S. Department of Agriculture***, *P.O. Box 5130, Madison, Wisc. 53705 (U.S.A.)*

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SUMMARY

A number of alkyl esters of resin acids were prepared with dialkyl acetals of dimethylformamide, and their gas chromatographic characteristics evaluated with several packed columns. The cyanosilicone liquid phases allow for simultaneous determination of levopimaric and palustric acids, a determination previously unattainable by gas–liquid chromatography.

INTRODUCTION

Hudy[1] in 1959 applied gas–liquid chromatography (GLC) to the analysis of resin acid methyl esters using Apiezon N and three polyesters. A number of workers have since extended that work to include a variety of other liquid phases. An overview of the field can be found in the reviews of Zinkel *et al.*[2] and Bardyshev and Bulgakov[3].

Although a number of gas chromatographic systems have been studied, satisfactory resolution of certain resin acid methyl esters has not been attained, such as in analyzing natural products containing levopimaric and palustric acids. Two possible approaches for satisfactory resolution are (1) increasing liquid phase polarity, and (2) accentuating the differences of the double-bond character of the resin acid esters by reducing the common leveling effect of the ester carbonyl–liquid phase interaction. The applicability of polar cyanosilicone liquid phases and hindered *tert.*-butyl esters in the GLC of resin acids to effect these approaches is the subject of this paper.

EXPERIMENTAL

Hewlett-Packard (Models 5750 and 5830) and Research Specialties (Model 600) gas chromatographs equipped with flame-ionization detectors (FIDs) were used. Column packings were prepared by the slurry techniques using Chromosorb W A-W

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* Maintained in cooperation with the University of Wisconsin.

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support and the liquid phase, followed by fluidized drying. Stainless-steel columns were used during acquisition of retention characteristics but were later replaced by glass columns. Columns were packed by both free-fall and pressure techniques appropriate for the column configuration.

Liquid phases included the methyl silicone SE-30 [on a support precoated with ethylene glycol isophthalate (EGiP)], the trifluoropropyl silicone SP-2401 (Supelco, Bellefonte, Pa., U.S.A.), the phenyl methyl silicone OV-17, Carbowax 20M, the phenyl cyanopropyl methyl silicone OV-225, diethylene glycol succinate (DEGS), and the cyanosilicones Silar 10C (Applied Science Labs., State College, Pa., U.S.A.) and OV-275.

Diazomethane, prepared from N-methyl-N-nitroso-*p*-toluenesulfonamide, was used to prepare the resin acid methyl esters[4]. Other resin acid esters were prepared with the dialkyl acetals of dimethylformamide (DMF). The ethyl, propyl, isopropyl, butyl, and *tert.*-butyl reagents were obtained from Pierce (Rockford, Ill., U.S.A.), the cyclohexyl reagent from Aldrich (Milwaukee, Wis., U.S.A.). To prepare esters with the dialkylacetal reagents, 10 mg of resin acids and 200 mg of the neat reagent were heated in a dry, capped vial at 60° overnight. The excess reagent was removed at reduced pressure with heating. The esters were dissolved in petroleum ether for GLC.

Resin acid methyl esters for determination of the response factor were purified by the methods required in preparing standards for reference spectra[2].

RESULTS AND DISCUSSION

Columns

In a previous study[5], DEGS was found the most generally useful liquid phase for the GLC of resin acid methyl esters (we have found the retention values on EGSS-X are almost identical with those for DEGS; the liquid phases are interchangeable for this purpose with the minor disadvantage of a smaller solute capacity with EGSS-X). However, the very long retention characteristics of other resin acid esters having additional oxygen-containing functionality (CHO, COH, COOMe) require a non-polar packing. SE-30 on a support precoated with a small amount of EGiP has been most useful in this respect[5]. The EGiP precoating considerably reduces support-catalyzed levopimarate decomposition, but does not eliminate it.

In earlier work in this laboratory, Anakrom ABS was found the most inert support available for GLC of resin acid methyl esters because it did not cause isomerization of palustrate and even more susceptible levopimarate. Over the years, however, the degree of Anakrom ABS silanization was increased by the manufacturer; this provided an improved support for many applications but concurrently resulted in an unsatisfactory support for preparation of efficient polyester packings. Fortunately, improvements in the preparation of Chromosorb W AW have provided an acceptable replacement.

In earlier observations at this laboratory on the effect of the column material we found that glass columns were of no advantage over stainless-steel columns containing DEGS packings when using the requisite amount of resin acid esters for an adequate thermal conductivity detector response. We have now verified this observation for an EGSS-X packing even at the much lower sample-size levels used with the FID.

This is not the case, however, with a cyanosilicone liquid phase such as Silar 10C. Extensive decomposition of the readily isomerizable methyl levopimarate can only be avoided by using glass columns. As a general precaution, glass columns are now used in the major portion of our GLC investigations involving resin acid esters.

Resin acid esterification

To facilitate gas chromatography of resin acids, the methyl ester is usually prepared. Although several reagents can be used to accomplish the methylation of the analogous fatty acids[6], only two methods, reaction with diazomethane and pyrolysis of the tetramethyl ammonium salt[7], can be used with the more hindered resin acid carboxyl group.

On comparing the functional groups of the various resin acid methyl esters (18-carboxylates) it seems reasonable that the carbomethoxy function will have interactions of the same magnitude for polar liquid phases. Hindered alkyl esters such as the trimethylsilyl ester, which is readily prepared, should accentuate the differences in unsaturation, thus, enhance the separations. Evidence from the retentions of the trimethylsilyl esters on several liquid phases of low to moderate polarity indicated, indeed, the approach was valid[8]. However, the trimethylsilyl esters are very susceptible to hydrolysis or transesterification during GLC on polar liquid phases (a fact overlooked in several publications on the GLC of trimethylsilyl esters of various acids).

Not until the relatively recent availability of the dialkyl acetal dimethylformamide reagents was there a potential for convenient preparation of a variety of other esters for analytical purposes. For example, saturated fatty acid esters are prepared rapidly and quantitatively by this method[9], although care must be exercised to avoid spurious GLC peaks[10]. We have found the reaction of resin acids with the neat reagent at 60° overnight is essentially quantitative. As an interesting aside, the phenolic hydroxyl as well as the axial carboxyl of podocarpic acid was found to be methylated by the dimethyl acetal reagent. The determination of the specific conditions for quantitative methyl ether formation for podocarpic acid or other phenolics was outside the scope of this investigation.

Methyl, ethyl, propyl, isopropyl, butyl, *tert.*-butyl, and cyclohexyl esters of pimaric acid were prepared using the dialkyl acetal–DMF reagents. Comparative retention data on eight liquid phases with a broad range of polarities are presented in Table I. Inspection of the data shows (1) the expected increase in retention with increasing molecular weight for the *n*-alkyl and cyclohexyl esters and (2) a pattern of decreasing retention with increasing liquid phase polarity for the more hindered isopropyl and *tert.*-butyl esters (the retention times for the isopropyl and *tert.*-butyl esters on the more polar liquid phases are less than that of the corresponding methyl ester). The retention characteristics for esters of the other usual pine resin acids (sandaracopimaric, palustric, levopimaric, isopimaric, abietic, dehydroabietic, and neoabietic acids) paralled the data in Table I for pimaric acid esters.

GLC separation of resin acid esters

Because of the possibility for improved separations coupled with decreased retention time, the *tert.*-butyl esters appeared a promising replacement for the methyl esters in the GLC of resin acids. Relative retention data for these two esters are compared for nine liquid phases (including the new polar phase, OV-275) in Table II.

TABLE I

GLC RETENTION CHARACTERISTICS OF ALKYL ESTERS OF PIMARIC ACID

Conditions			*Retention time of methyl ester (min)*	*Retention time relative to methyl ester*					
Liquid phase	*Column temp. (°C)*	*Nitrogen flow-rate (ml/min)*		*Ethyl*	*n-Propyl*	*n-Butyl*	*Cyclo-hexyl*	*Iso-propyl*	*tert.-Butyl*
9% SE-30-EGiP (1/8 in. × 6 ft.)	200	90	10.4	1.20	1.63	2.18	5.32	1.27	1.35
3% SP-2401 (1/8 in. × 10 ft.)	200	35	8.6	1.15	1.54	2.07	4.72	1.27	1.20
3% OV-17 (1/8 in. × 6 ft.)	210	40	6.1	1.16	1.54	2.08	5.32	1.12	1.14
3% Polyphenyl ether (6-ring)	210	35	9.6	1.13	1.54	2.18	6.56	1.04	1.01
5% OV-225 (1/4 in. × 6 ft.)	210	70	8.9	1.09	1.41	1.77	4.53	1.00	0.97
5% Carbowax 20M (1/8 in. × 6 ft.)	210	20	8.5	1.06	1.35	1.72	4.59	0.94	0.87
10% DEGS (1/8 in. × 8 ft.)	200	50	21.0	0.98	1.23	1.52	4.04	0.86	0.75
10% Silar 10C (1/8 in. × 11 ft.)	200	20	21.8	1.00	1.23	1.49	3.70	0.83	0.73

TABLE II

GLC RETENTION CHARACTERISTICS OF METHYL (Me) AND *tert.*-BUTYL (t-Bu) ESTERS OF RESIN ACIDS AT 200°

Liquid phase	*Ester*	*Resin acid*							
		Pimaric	*Sandaraco-pimaric*	*Levo-pimaric*	*Palustric*	*Iso-pimaric*	*Abietic*	*Dehydro-abietic*	*Neo-abietic*
SE-30–EGiP	Me	1.00	1.06	1.22	1.22	1.20	1.55	1.34	1.84
	t-Bu	1.00	1.05	1.24	1.17	1.09	1.41	1.27	1.82
SP-2401	Me	1.00	1.08	1.21	1.17	1.28	1.60	1.39	1.77
	t-Bu	1.00	1.09	1.21	1.11	1.16	1.41	1.34	1.77
OV-17	Me	1.00	1.07	1.33	1.31	1.28	1.74	1.67	2.17
	t-Bu	1.00	1.08	1.32	1.31	1.17	1.63	1.62	2.20
PPE	Me	1.00	1.09	1.38	1.39	1.39	2.08	2.01	2.57
	t-Bu	1.00	1.10	1.37	1.37	1.23	1.91	1.98	2.55
OV-225	Me	1.00	1.08	1.29	1.27	1.29	1.75	1.69	2.12
	t-Bu	1.00	1.09	1.30	1.22	1.19	1.41	1.65	2.12
Carbowax 20M	Me	1.00	1.10	1.34	1.36	1.39	2.01	2.04	2.40
	t-Bu	1.00	1.09	1.33	1.33	1.22	1.83	1.92	2.38
DEGS	Me	1.00	1.12	1.33	1.34	1.46	2.08	2.25	2.46
	t-Bu	1.00	1.11	1.33	1.31	1.25	1.86	2.15	2.39
Silar 10C	Me	1.00	1.15	1.42	1.36	1.51	2.13	2.48	2.53
	t-Bu	1.00	1.14	1.40	1.31	1.32	1.87	2.30	2.42
OV-275[*]	Me	1.00	1.15	1.38	1.29	1.54	2.14	2.56	2.54
	t-Bu	1.00	1.15	1.46	1.27	1.35	1.92	2.37	2.49

[*] Column of 1/8 in. × 10 ft. packed with 20% OV-275 on 80–100 Chromosorb W AW at 200°; nitrogen flow-rate, 17 ml/min. Retention time of methyl ester, 15.0 min.

Although improved separation is seen for *tert.*-butyl levopimarate and palustrate with several of the packings, overlap of other previously resolved components now occurs.

Previous to our work with OV-275, we used the methyl and *tert.*-butyl esters for determining the palustric and the levopimaric acid contents in pine extractives by GLC analysis on Silar 10C but GLC analysis on another (DEGS or EGSS-X) column was necessary to resolve the methyl dehydroabietate–neoabietate or the *tert.*-butyl palustrate–isopimarate overlaps. The separations shown in Table II for the *tert.*-butyl esters of the common resin acids indicate that OV-275 would be the liquid phase of choice. However, we and others[11] have not been able to obtain as efficient columns with OV-275 using Chromosorb W supports as with Silar 10C and other liquid phases. In addition, we have observed that the 15% OV-275 on Chromosorb P AW DMCS column packings reported[12] to be efficient for resolution of fatty acid esters such as methyl oleate–elaidate cause complete decomposition of levopimarate and very extensive decomposition of palustrate. The longer time in preparation of the *tert.*-butyl ester by the *tert.*-butyl acetal of DMF reagent compared with the quick preparation of the methyl ester with diazomethane also can be an important factor in a specific analytical situation. Thus we are now analyzing the resin acids in most pinewood extractives and oleoresins as their methyl esters with a two-column GLC system using 10% EGSS-X for the primary data and 20% OV-275 for the determination of methyl levopimarate and palustrate.

Routine GLC of resin acid esters on polyester liquid phases has been limited to column temperatures of about 200° because of liquid phase instability. The greater stability of the cyanosilicones, however, permits an evaluation of possible improved separation at higher temperatures not only for palustrate–levopimarate, but also for other less common and difficultly resolved pairs such as communate–8,13β-epoxy-14-labden-19-oate (found in *Pinus resinosa* needles[13]) and anticopalate–isopimarate[14]. Little change is seen, however, in separation of the resin acid methyl esters with increasing column temperature (Table III) except for some small improvement in the dehydroabietate–neoabietate pair. Thus, GLC on two, and in some cases even three

TABLE III

EFFECT OF TEMPERATURE ON RETENTION OF RESIN ACID METHYL ESTERS ON CYANOSILICONE LIQUID PHASES

Methyl ester	*10% Silar 10C*			*20% OV-275*		
	200°	*215°*	*230°*	*200°*	*215°*	*230°*
Pimarate	1.00	1.00	1.00	1.00	1.00	1.00
Sandaracopimarate	1.16	1.14	1.12	1.15	1.14	1.13
Levopimarate	1.42	1.38	1.34	1.38	1.34	1.30
Palustrate	1.37	1.32	1.26	1.29	1.24	1.20
Communate	1.40	1.34	1.27	1.38	1.31	1.25
8,13β-Epoxy-14-labdenoate	1.39	1.35	1.31	1.41	1.36	1.32
Anticopalate	1.48	1.42	1.37	1.41	1.34	1.31
Isopimarate	1.53	1.49	1.44	1.54	1.50	1.46
Abietate	2.17	2.05	1.92	2.14	2.02	1.90
Dehydroabietate	2.54	2.35	2.15	2.50	2.27	2.11
Neobaietate	2.57	2.42	2.24	2.54	2.35	2.21

(*e.g.*, OV-275, EGSS-X and SE-30), columns is necessary to obtain complete analyses for the individual resin acids in pine extractives.

Response factors

The relative response, calculated as correction factors[5], of resin acid methyl esters are compared in Table IV with data reported by Pensar and Bruun[15]. The large correction factors, reported by Pensar and Bruun most likely reflect the state of purity of their samples; the resin acids, especially the abietadienoic acids (and methyl esters), are readily oxidized by air.

TABLE IV

FID CORRECTION FACTORS FOR RESIN-ACID METHYL ESTERS

Methyl ester	*Correction factor*	
	Present work	*Calculated from data ref. 15*
Pimarate	1.00	1.00
Sandaracopimarate	1.03	1.03
Palustrate	1.05	1.17
Levopimarate	1.07	—
Isopimarate	0.99	1.00
Abietate	1.08	1.15
Dehydroabietate	0.99	1.09
Neoabietate	1.12	1.31

CONCLUSIONS

The highly polar cyanosilicone liquid phases, Silar 10C and OV-275, are useful in the GLC of both methyl and *tert.*-butyl esters of diterpene resin acids, particularly in the separation of palustrate–levopimarate. No single liquid phase in a packed column, however, provides sufficient separation between the common pine resin acids for an adequate resolution of all components. Thus, the complete analysis of all the resin acids from a pine extract requires GLC on several different columns.

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