Quantitative Separation of Diazelaoglycerides From Insoluble Azelaoglycerides and Its Applications in Glyceride Structure Studies

Abstract

Diazelains can be quantitatively removed from mixtures of di- and monoazelains by treatment in diethyl ether with saturated aqueous sodium bicarbonate under specified conditions. The total monoazelains produced by oxidation of GS_3nil fats of C_{16}–C_{18} acid type can be isolated quantitatively for further examination by applying this procedure to the insoluble azelaoglycerides. Similar treatment of the insoluble azelaoglycerides from GS_3 positive fats will give a mixture of GS_3 and GS_2A in the bicarbonate insoluble fraction. The GS_3 can be accurately estimated from the analysis of the latter by already available procedures.

Quantitative separation of different glyceride types, followed by investigation of individual types, is the ideal method for studying the structure of natural fats. The same result is achieved when the different types are converted to derivatives and the type derivatives are quantitatively separated. One such line of investigation has at last met with some success and a preliminary account follows. Acetic acid-acetone-permanganate oxidation converts fats quantitatively to mixtures of GS_3, GS_2A, GSA_2, GA_3 and lower acids (1) without any selective hydrolysis of dibasic acids in the azelaoglycerides (1) and also, as has recently been shown (2), without any over-oxidation of the latter as well. The improved azelaoglyceride separation (3,4) divides the oxidation products into two fractions: (a) Insoluble Azelaoglycerides (IAG) which contain all the GS_3, GS_2A, and almost all the GSA_2 in case of fats with saturated acid (S) content above 30%, or fats with mean M.W. of S above 285 and (b) Soluble Azelaoglycerides (SAG) which contain all the GA_3 along with a little GSA_2, in some cases and some residual lower saturated acids, as mentioned above. It has since been found that this separation is still more effective if the precipitated mixture is cooled at 5–10 C for 72 hr and then filtered cold, or kept at room temperature (18–30 C) for 72 hr and then chilled at 5–10 C for 4 hr before filtration. The actual proportions of GSA_2 then left behind in the soluble magnesium salt filtrates are as follows: (a) 0.0–0.5% in case of fats where S has mean M.W. above 285, regardless of Sm; (b) 0.0–1.0% in case of fats with Sm above 30, regardless of mean M.W. of S; and (c) 0.5–3.0% and 1.0–5.0% for fats with Sm 20 to 30 and mean M.W. of S between 285 to 270 and 270 to 255, respectively. In case of fats of the (c) group, the GSA_2 is minimal when the Sm is about 30 and gradually increases as Sm decreases. All the GSA_2, except about 1%, can be recovered in such cases by reprecipitation after washing the recovered SAG with 3% sodium bicarbonate solution to remove GA_3 (4). In the case of most of the fats of (a) and (b), the SAG filtrates give just 2–3 mg of S regardless of the weight of the fat used, indicating a very small limiting solubility of 2 mg GSA_2/100 ml filtrate. As a result, the IAG will now contain all the GS_3 and GS_2A and practically all the GSA_2, and the SAG will be a mixture of GA_3 with some residual lower acids and traces of GSA_2. The total GA_3 can be quantitatively isolated in the aqueous portions, when a solution of SAG in diethyl ether is shaken with freshly prepared (neutral to phenolphthalein) 3% aqueous solution of sodium bicarbonate in which the residual lower saturated acids are practically insoluble. Or it can be isolated by distillation of the SAG with steam, when the lower acids are volatilized leaving the GA_3 as residue.

Fractional crystallization or chromatographic procedures for separation of GS_2A and GSA_2 isolate some quantities of both in the pure state, but sizable proportions of intermediate fractions, depending on the complexity of the saturated and dibasic (5) acid fractions are left unresolved and these procedures do not effect quantitative separation of type derivatives even when only two types are present. The GSA_2 can, however, be quantitatively separated from the IAG by the following chemical procedure. The IAG (about 1 g) is dissolved in diethyl ether to give 1% to 3% solution and shaken well four times with freshly prepared, (neutral to phenolphthalein) saturated solution of sodium bicarbonate. Ten to 12 ml of solution containing 1 g bicarbonate are used each time and each bicarbonate shaking is followed by one washing with an equal amount of water. Emulsions formed are allowed to break entirely by standing; addition of alcohols or salt solutions for this purpose seriously interferes with the separations. After the bicarbonate shakings are over, the ether solutions are washed three times more with water. All washings are reextracted once with ether. Removal of solvent from the washed ether solutions gives the Bicarbonate Insoluble portion of IAG (BIIAG). The Bicarbonate Soluble portion of IAG (BSIAG) is recovered as usual.

In GS_3 nil fats of C_{16}–C_{18} acid type, the BIIAG will consist almost exclusively of GS_2A which can be established from the S content determined as usual (1); the latter should be within 0.5% to 1.0% of that theoretically required for known values of S and A. In case there is a significant amount of nonglyceridic matter, the BIIAG is to be analyzed in detail as described earlier for IAG (4). The BSIAG in case of C_{16}–C_{18} acid fats would be nearly pure GSA_2 in all cases since nonglyceridic matter does not, as a rule, accumulate in this fraction. S contents of this fraction higher than required by theory may be caused by imperfect separation of emulsions or too high pH for bicarbonate solutions and may be rectified by repeat separation of BSIAG as before. However BSIAG from fats containing large amounts of lower saturated acids tends to contain appreciable GS_2A since lower acid GS_2A is some-

what soluble in saturated bicarbonate solutions. The BIIAG will, however, be free from GSA_2 in all cases. BIIAG and BSIAG fractions corresponding to nearly pure GS_2A and GSA_2 (S content within 0.5% to 1.0% of that theoretically required for specific values of S and A) have been isolated in nearly theoretical (within ±1.0% on fat basis) yields [as computed from analyses of the IAG (4)] from IAG specimens of the following seed fats in this laboratory (Kartha and Ali, unpublished observations): *Garcinia indica,* Sm 66; *Vateria indica,* Sm 59; *Garcinia cambogia,* Sm 55; *Sarcestigma kleinii,* Sm 43; *Madhuca latifolia,* Sm 47; *Azadirachta indica,* Sm 33 to 40; and *Erythrina indica,* Sm 30 to 34.

In the case of fats containing GS_3, the BIIAG will be a mixture of GS_2A with all the GS_3 present in the fat and the proportions of GS_3 can be calculated from the theoretical S contents of GS_3 and GS_2A and the corrected S content of the fraction determined according to procedures described earlier (4).

The quantitative separation of GSA_2 from IAG serves two important purposes: (a) A large majority of vegetable and animal fats do not contain any GS_3, and in these cases complete separation of individual glyceride types as type derivatives is now possible. (b) So far it has not been possible to estimate the proportions of GS_3 present in oxidation products from fats except in the case of lower saturated acid fats (6,7) and even this is on a provisional basis. The present procedure makes possible a reasonably accurate oxidative estimation of GS_3 for any fat regardless of saturated acid composition or saturated acid content. The procedure will be particularly useful for analysis of fats which do contain appreciable amounts of GS_3 as it is more convenient than crystallization or chromatographic procedures and further, is not liable to error caused by autoxidative deterioration of unsaturated glycerides to iodine value free products.

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Unusual Resin Acids in Tall Oil[1]

Abstract

Combinations of silver nitrate column, gel permeation and gas liquid chromatography were used to isolate 8,15-pimaradien-18-oic, 8,15-isopimaradien-18-oic and the secodehydroabietic, 2α-[2′(*m*-isopropylphenyl)ethyl]1β,3α-dimethylcyclohexanecarboxylic acids as their methyl esters from a fraction of distilled tall oil.

Gas chromatographic examination of an intermediate fraction from the distillation of tall oil revealed that the fraction contained resin acids other than the standard abietic and pimaric type acids usually found in rosin. Although the constituents are minor components of tall oil itself, their enrichment in certain tall oil products can have an important effect on the end uses of these products.

In our work on the separation and purification of rosin acids for use as primary standards for GLC and spectral use, a combination of silver nitrate column chromatographic (as used to purify the abietenoates) (1) and preparative GLC methods has been useful. Multibore columns (15 × 1.2, 15 × 1.9, 19 × 2.6 and 15 × 3.5 cm stages) containing 200 g of silver nitrate-Woelm neutral alumina (4:10) were used in all column chromatographic separations. Petroleum ether with ethyl ether in increasing steps was used as the eluting solvent.

A 20 g fraction of methyl esters of an intermediate fraction from a tall oil distillation (the bulk of the fatty acids had been removed by preferential esterification) was placed on the column in an equal volume of petroleum ether. The eluate was monitored by GLC and appropriately combined as follows in order of increasing amount of ethyl ether in the eluant: Fraction I, 6.3 g; Fraction II, 1.8 g; Fraction III, 5.2 g; Fraction IV, 0.3 g; Fraction V, 2.1 g; Fraction VI, 1.8 g; and Fraction VII, 0.6 g. Fractions II, IV and VI were similar to the neighboring fraction, but were not as enriched in components of current interest; they were not investigated further. Fraction VII was shown by GLC (2) to contain methyl *cis*-5, *cis*-11, *cis*-14-eicosatrienoate as its major component, a common constituent in tall oil (3).

On the basis of the relative retention on DEGS and SE-30 columns, (2), the major constituent of the intermediate tall oil fraction was suspected to be methyl Δ^8-isopimarate [methyl 8,15-isopimaradien-18-oate. The numbering and systematic nomenclature follow the recent proposals by J. W. Rowe et al. (4)]. Fraction V was recrystallized from methanol and was indeed shown to be nearly pure methyl Δ^8-isopimarate by comparison of the NMR, IR and GLC spectra with those for authentic material. Δ^8-Isopimaric acid has been identified as a minor constituent of pine oleoresin (5,6) and tall oil rosin (6).

Rechromatography of a 2.5 g portion of Fraction III on a new silver nitrate-alumina column yielded more Δ^8-isopimarate and about 500 mg of a second major component in high purity. Recrystallization from methanol gave pure material (GLC) (2) of mp 37.5–38 C, cor. The postulated identity of this second component as methyl Δ^8-pimarate [methyl 8,15-pimaradien-18-oate) (4)] based on comparative GLC retention data (2), was confirmed by comparison of the NMR with the data reported by ApSimon (6). The presence of Δ^8-pimaric acid in naval stores products has not been reported, although Genge (7) postulated its presence on mass spectral evidence.

Rechromatography of Fraction I on silver nitrate-

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