Separation and Purification of Pinolenic Acid by the Iodolactonization Method

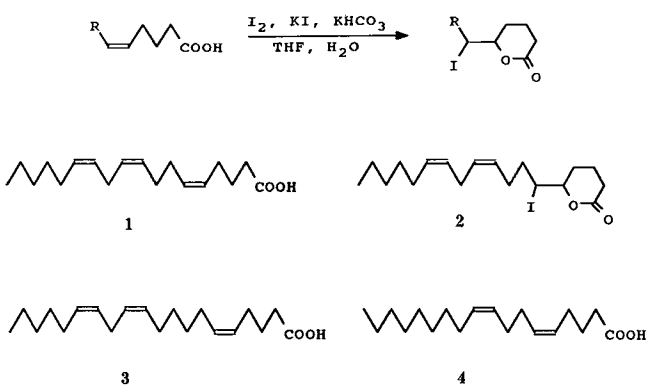
Anneli Hase[a,*], Maija Ala-Peijari[a], S. Kaltia[b] and J. Matikainen[b]

[a]Technical Research Centre of Finland, Chemical Laboratory, SF-02151 Espoo, Finland and [b]University of Helsinki, Department of Chemistry, SF-00100 Helsinki, Finland

The separation of pinolenic acid from distilled tall oil fatty acid by the iodolactonization method was studied. The significance of prefractionation of tall oils and details of the iodolactonization procedure for the pinolenic acid purity achieved is discussed.

KEY WORDS: Fatty acid, iodolactonization, pinolenic acid, purification, separation, tall oil, 5,9,12-octadecatrienoic acid.

Scandinavian distilled tall oil fatty acid (TOFA) contains more than 10% pinolenic acid (1) (5Z,9Z,12Z-octadecatrienoic acid (1,2). Pinolenic acid can be prepared from TOFA by urea fractionation (3) and by countercurrent argentation (4). Pinolenic acid of high purity can be prepared from TOFA by combining these two techniques (5). Recently (6; A. Hase, S. Kaltia, J. Matikainen, M. Ala-Peijari and T. Hase, submitted for publication; 7), thermodynamic properties of pinolenic acid cyclizates, *i.e.* the isomers of 4-(5-pentyl-3a, 4,5,7a,-tetrahydro-4-indanyl)butanoic acid, and the corresponding esters have been determined. It has been shown that the cyclizate esters do not crystallize and that they have interesting thermal properties at subambient temperatures (6 and A. Hase, S. Kaltia, J. Matikainen, M. Ala-Peijari and T. Hase, submitted for publication). The present study has been done to elucidate the possibility of using the iodolactonization method, earlier successfully used in purification of arachidonic (8,9) and docosahexaenoic acids (10), for the preparation of pure pinolenic acid from TOFA (Scheme 1).



SCHEME 1

EXPERIMENTAL PROCEDURES

Materials. TOFA, containing 11% pinolenic acid, *ca.* 1% 5Z,11Z,14Z-eicosatrienoic acid (3), *ca.* 1% 5Z,9Z-octadecadienoic acid (4) and *ca.* 2% resin acids and *ca.* 2% neutral material, was obtained from Veitsiluoto Oy, Oulu, Finland. Tetrahydrofuran (THF), dichloromethane (DCM) and acetonitrile (ACN) were high-performance liquid chromatography (HPLC)-grade from Rathburn Chemicals Ltd., Walkerburn, Scotland. Chlorotrimethylsilane (CTMS) was from Fluka A.G., Buchs, Switzerland, *bis*(trimethylsilyl)-trifluoroacetamid (BSTFA) for gas chromatography and all other chemicals were p.a. grade from E. Merck, Darmstadt, Germany. ACN was dried over molecular sieve 564 (3 Å) from Grace GmbH, Worms, Germany.

Urea fractionation as described earlier (3) was performed to prepare a 57% pinolenic acid methyl ester concentrate from TOFA. This concentrate contained 19% linoleic acid methyl ester, 6% 5Z,11Z,14Z-eicosatrienoic acid (3) methyl ester and 5% 5Z,9Z-octadecadienoic acid (4) methyl ester according to mass spectrometry and gas-liquid chromatography (GLC) analysis with a BDS column.

Analysis. GLC was performed on a Hewlett-Packard 5890 instrument equipped with a flame-ionization detector and a Hewlett-Packard 3396A integrator (Palo Alto, CA). The two columns used were a 25 m × 0.32/0.44 mm NB-1 silica capillary column and a 50 m × 0.27/0.82 mm BDS glass capillary column. Mass spectra were recorded by GLC/mass spectrometry (MS) with a JEOL SX-102 instrument (Tokyo, Japan). The nuclear magnetic resonance (NMR) spectra were run on a Varian (Palo Alto, CA) Gemini 200 spectrometer (200 MHz for ^1H).

Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} and column chromatography (CC) was performed with silica gel 60 (0.2 to 0.5 mm), both from E. Merck.

Lactonization and regeneration of the fatty acids. Pinolenic acid purification *via* the corresponding iodolactone (2) was performed according to Corey *et al.* (8,9). The lactone formation was started by dissolving 16.1 g TOFA in an aqueous potassium hydrogen carbonate solution (25 mL, 20%) containing THF (45 mL) and potassium iodide (5.73 g) at a temperature of −1°C. Iodine (16.6 g) was added to this TOFA solution during 40 min while stirring with a magnetic stirrer. The reaction was completed by stirring the mixture for 19 h at a temperature of −2°C to +6°C. The excess iodine was destroyed with 100 mL of chilled sodium thiosulfate solution (46%) at a temperature of 6°C. The iodolactone was extracted from the solution with 100 mL of pentane:diethylether (3:2) mixture at 6°C. Extraction was performed three times, and the combined extracts were washed with 2 × 100 mL solution of sodium carbonate (3.5%) and 2 × 60 mL of saturated sodium chloride solution at 6°C. The extract was dried with anhydrous sodium sulfate, and the solvents were evaporated in vacuum at a temperature of 35°C. The yield of iodolactone was 1.64 g.

Part of the iodolactone (0.223 g) was dissolved in 3 mL of dry ACN and regenerated to fatty acids by a suspension of dried sodium iodide (0.4 g) in anhydrous ACN (3 mL). The lactone-iodide-ACN solution was stirred 10 min in a vessel protected from ambient moisture by a tube

*To whom correspondence should be addressed at Technical Research Centre of Finland, Chemical Laboratory, P.O. Box 204, SF-02151 Espoo, Finland.

filled with anhydrous calcium chloride. CTMS (0.1 g) was added, and the mixture was stirred for 1 h while protected from light and moisture at ambient temperature. After addition of sodium thiosulfate solution (7 mL, 10%) and a short stirring, the regenerated fatty acids were extracted from the solution with 4 × 2.5 mL of hexane:DCM mixture (3:1). The extract was washed with 4 × 2 mL of water and 2 × 2.5 mL of saturated sodium chloride solution. The extract was dried with anhydrous magnesium sulfate and evaporated. The yield of the fatty acids was 0.145 g. A sample of the fatty acids was methylated by means of diazomethane or silylated by using CTMS and BSTFA in THF before GLC analysis.

Part of the iodolactone (1.40 g) was purified by CC on 100 g of silica gel and by eluting the lactone with diethylether:hexane (3:1) mixture as the eluent. The elution should be performed quickly to minimize reactions during the chromatography. In 30 min, 640 mL of eluent was collected in 30-mL fractions. The eluent was monitored by TLC in which DCM was used as the eluent and iodine vapor for staining the TLC plates. The iodolactone was eluted in fractions between 120 and 240 mL, which were combined and dried over sodium sulfate. After evaporation of the solvent mixture, 0.5 g of purified iodolactone was obtained. This purified lactone was regenerated to fatty acids as described above. After regeneration, 0.18 g of fatty acids was obtained.

The iodolactonization method was also applied to the 57% pinolenic acid concentrate of TOFA. When 1.00 g of this starting material was used and the lactonization procedure was performed as described above, 0.77 g of iodolactone was obtained. After the CC purification of this iodolactone, 0.62 g of purified iodolactone was obtained. ^{1}H NMR: δ 0.99 (*t*, J = 6.5 Hz, 3H, H-18), 1.20–1.85 (*m*, 18H), 1.92 (*t*, J = 7.3 Hz, 2H, H-11), 4.18 (*m*, 1H, H-6), 4.22 (*m*, 1H, H-5), 5.35 (*m*, 4H, H-9, H-10, H-12, H-13). ^{13}C NMR: δ 14.3, 18.6, 22.8, 26.1, 27.4 (2C), 27.5, 29.5, 29.7, 31.7, 35.7, 38.1, 82.5, 127.5, 127.7, 130.4, 130.8, 170.8. Regeneration gave 0.36 g of fatty acids from the 0.62 g of purified iodolactone.

Experiments were also made to learn the importance of adding iodine slowly in the formation of iodolactone and the effect of moisture in ACN in the regeneration of pinolenic acid from the iodolactone. In these experiments, iodine was added in less than 1 min to the THF-KI solution of pinolenic acid and undried p.a. grade ACN (from E. Merck) was used in the regeneration step. Pinolenic acid 57% concentrate was used as the starting material.

Removing sulfur. The iodolactone samples and regenerated fatty acid (FA) samples contained sulfur from 0 to 30%. The sulfur contaminating the samples originates from the thio-sulfate solution used in destroying excess iodine in preparation of the iodolactone, and also used in regeneration of the iodolactone. Sulfur was removed by dissolving the sample in 64% aqueous ethanol solution and filtering the solution. The filtrate was diluted with water to make it 10% ethanol and the FA was extracted to hexane. After drying the hexane solution with anhydrous sodium sulfate, the solvent was evaporated and a sulfur-free sample was obtained.

RESULTS AND DISCUSSION

Iodolactones are only formed from carboxylic acids that have a double bond in position 4 or 5. In addition to pinolenic acid, **3** and **4** are iodolactone-forming compounds in TOFA and are present in small concentration. According to the present study, iodolactonization is an excellent method for preparing pinolenic acid from TOFA, especially when pinolenic acid concentrate is available as the starting material. When TOFA is used as the starting material, the efficiency of the lactone separation tends to be lowered due to the massive soap formation from fatty acids other than pinolenic acid. The purity of pinolenic acid prepared from TOFA by iodolactonization was 80%, and that from the concentrate was 96%. The numbers include the unavoidable acids **3** and **4** (12 to 13% together in both cases).

CC purification of iodolactone leads to a product with somewhat higher purity by removing the unreacted fatty acids. Iodolactone reacts during chromatography, thus reducing the yield. When iodolactone is prepared from pinolenic acid concentrate, purification of the lactone by CC is unnecessary. When TOFA is the starting material, soap formation tends to bring more impurities into the preparation. The purity can then be improved by CC of the iodolactone.

Moisture in ACN during regeneration of pinolenic acid from the corresponding iodolactone causes the formation of compounds with one iodine atom added in the fatty acid chain. This result was confirmed by GC/MS. Up to 6% of the iodinated fatty acid was determined in the sample prepared with undried ACN. If iodine is added too rapidly in the preparation of iodolactone, a product of lower purity is also obtained.

During the preparation of this manuscript, a paper was published presenting data for optimizing the solvents, time, and ratio of KI/I_2 in the iodolactonization of sardine oil fatty acids and rat liver fat (11). The authors used higher temperatures in the iodolactonization (13°C and 25°C) than used here. The solvents used were ethanol and 1,4-dioxane in addition to THF as used here. Ethanol was recommended as giving the fastest formation of iodolactone. That paper did not discuss the purity of the products obtained from the point of view of the addition of iodine to the fatty acid chain. The time, solvent and temperature (−1° to +6°C) used in our study were sufficient for quantitative lactone formation as can be seen from the high yield of the lactone.

When pure pinolenic acid is prepared from TOFA, iodolactonization is a good method, especially when combined with some prepurification method, *i.e.* urea fractionation or solvent crystallization. Iodolactonization does not separate pinolenic acid from **3** and **4**, but enables a one-step preparation of pinolenic acid with mainly or only **3** and **4** as impurities, depending on whether tall oil or a preconcentrate is used as starting material. When preparing pinolenic acid of highest purity, **3** can be removed by careful distillation and **4** by countercurrent argentation fractionation.

ACKNOWLEDGMENTS

Maija Ala-Peijari was economically supported by a research grant from Emil Aaltonen Foundation for this work. Anja Salakari provided valuable technical assistance.

REFERENCES

1. Aho, Y., O. Harva and S. Nikkilä, *Tek. Kemian Aikak. 19*:390 (1962).

2. Lehtinen, O., V. Kärkkäinen and M. Antila, *Suomen Kemistilehti B 35*:179 (1962).
3. Hase, Anneli, O. Harva and T. Pakkanen, *J. Am. Oil Chem. Soc. 51*:181 (1974).
4. Scholfield, C.R., R.O. Butterfield and H.J. Dutton, *Anal. Chem. 38*:1694 (1966).
5. Hase, Anneli, O. Harva and V. Jaakkola, *Kem. Teollisuus 29*:885 (1972).
6. Hase, Anneli, Finnish Chemical Congress 30.10.-1.11.1990, Poster 5.43, ref. *Kemia-Kem. 17*:10B:975 (1990).
7. Hase, A., and P. Saikkonen, *Fat Sci. Technol. 93*:112 (1990).
8. Corey, E.J., and S.W. Wright, *Tetrahed. Lett. 25*:2729 (1984).
9. Corey, E.J., and Shun-ichi Hashimoto, *Ibid. 22*:299 (1981).
10. Wright, S.W., E.Y. Kuo and E.J. Corey, *J. Org. Chem. 52*:4399 (1987).
11. Gaiday, N.V., A.B. Imbs, D.V. Kuklev and N.A. Latyshev, *J. Am. Oil Chem. Soc. 68*:230 (1991).

[Received October 29, 1991; accepted April 21, 1992]