Phytochemistry, 1969, Vol. 8, pp. 2219 to 2222. Pergamon Press. Printed in England.

SEED LIPIDS OF THE AMERICAN CRANBERRY (*VACCINIUM MACROCARPON*)

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(*Received* 13 *May* 1969)

Abstract—The lipids from cranberry seeds have been shown to contain phosphatidyl choline, serine and ethanolamine, mono- and digalactosyl glycerides and a number of neutral components including glycerides, hydrocarbons and several triterpenoids. Glyceride fatty acids were determined, and the hydrocarbon and triterpenoid fractions examined in further detail.

INTRODUCTION

THE LIPID composition of the American Cranberry (*Vaccinium macrocarpon* Ait.—Ericaceae), an important Massachusetts agricultural commodity, has not been well characterized. The volatiles from this fruit have recently been investigated[1,2] and the cuticular lipids are currently under investigation in this laboratory. A number of earlier investigators[3–6] have examined the lipids extracted from cranberry press-cake (the seed and skin residue remaining after juice expression) and noted the presence of glycerides,[3] nonacosane,[3] hentriacontane,[3] alcohols,[5] ursolic acid[3–5] and oleanolic acid.[5,6] It is difficult to ascertain, from this earlier work, the seed lipid contribution to press-cake extracts.

No reference to the *Vaccinium* seed oils is contained in the compendia of Eckey,[7] Hilditch,[8] or Wolff and co-workers.[9] A single reference to cranberry seed oil was made, however, by Nealy[10] in which the oil was described as semidrying with a greenish-yellow color and a bland taste, but no chemical analysis was presented. This paucity of information, coupled with a desire to continue working with this fruit, prompted the investigation of the seed lipids.

RESULTS AND CONCLUSIONS

An isopropanol–$CHCl_3$ extract[11] of dry crushed seeds was purified by Sephadex column chromatography[12] and yielded 23·3 per cent lipid (20 per cent according to Nealy[10]). The lipid extract was separated into three fractions by silicic acid column chromatography using

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$CHCl_3$ (neutral lipids), acetone (glycolipids) and methanol (phospholipids) as the eluting solvents.[13]

The neutral fraction comprised 95·5 per cent of the lipid extract, and was shown by TLC on silicic acid, followed by charring and densitometry,[14] to contain hydrocarbons, 12 per cent; triglycerides, 53 per cent; diglyceride, 12 per cent; monoglyceride, 7 per cent; sterol, 6 per cent; pentacyclic triterpene alcohol, 3 per cent; and triterpene acid, 8 per cent. Neutral lipids were saponified and the non-saponifiable portion subjected to preparative TLC whereby hydrocarbon, pentacyclic triterpene alcohol and sterol subfractions were isolated.

Gas chromatography of the hydrocarbon subfraction revealed the presence of *n*-C_{16}, 18 per cent; *n*-C_{18}, 20 per cent; *n*-C_{20}, 12 per cent; *n*-C_{22}, 3 per cent; *n*-C_{24}, 21 per cent; and squalene, 23 per cent. Minor amounts of C_{15}, C_{17}, C_{19}, C_{21}, C_{23}, C_{26}, C_{28}, C_{29}, and C_{31} were also present.

The pentacyclic triterpene alcohol subfraction was converted to the trimethylsilyl (TMS) ethers[15] and shown by GLC to consist of α-amyrin, 75 per cent of fraction; β-amyrin, 22 per cent; and another unidentified alcohol, 3 per cent. The sterol subfraction was treated similarly[15] and shown to contain β-sitosterol, 71 per cent; stigmasterol, 12 per cent; campesterol, 12 per cent; and an unidentified sterol, 5 per cent. Retention of the unknown was 1·11 relative to β-sitosterol.

The saponifiable portion of the neutral lipids was subjected to preparative TLC and fatty acid and triterpene acid subfractions isolated. The fatty acids were methylated,[16] and on analytical GLC gave rise to the results reported in Table 1. The triterpene acid subfraction was converted to the TMS-ether, TMS-ester derivatives,[15] and on GLC shown to consist of ursolic acid, 85 per cent of subfraction; oleanolic acid, 15 per cent; and a trace of an unidentified acid of retention 1·52 relative to ursolic acid.

The glycolipid fraction comprised 3·4 per cent of the lipid extract. On TLC this fraction yielded monogalactosyl diglyceride, 80 per cent; digalactosyl diglyceride, 15 per cent; unknown R_f 0·67, 5 per cent; and a trace of unknown R_f 0·18. The glycolipid fraction was methylated[16] and the fatty acid methyl esters identified by GLC (Table 1).

TABLE 1. FATTY ACID COMPOSITION OF CRANBERRY SEED LIPID FRACTIONS (EXPRESSED AS AREA PERCENTAGES OF TOTAL METHYL ESTERS)

n-Fatty acids	Neutral lipids (%)	Glycolipids (%)	Phospholipids (%)
C_{14} and shorter	trace	3	1
$C_{16:0}$	7	32	3
$C_{16:1}$	trace	3	1
$C_{18:0}$	trace		
$C_{18:1}$	30	40	59
$C_{18:2}$	33	15	27
$C_{18:3}$	30	6	10

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The phospholipid fraction comprised 1·1 per cent of the lipid extract and TLC analysis indicated the presence of phosphatidylcholine, 90 per cent; phosphatidylserine, 5 per cent phosphatidylethanolamine, 5 per cent; and a trace of lysophosphatidyl choline. The fatty acid composition was determined as above for glycolipids (Table 1).

The presence of triterpenoids in *Vaccinium macrocarpon* seeds is not surprising as the genus *Vaccinium* have been previously reported as being rich in triterpene materials.[17] A number of triterpenes including α-amyrin, β-amyrin, β-sitosterol, ursolic acid and oleanolic acid have previously been isolated from leaf,[18–20] and root extracts[21] of *Vaccinium* species other than *macrocarpon*. Laakso[5] has previously identified ursolic and oleanolic acids in the press-cake extracts of this fruit. It has now been shown that triterpenes comprise a significant portion of *V. macrocarpon* seed lipids.

Earlier investigators[3] have also reported the presence of glycerides in cranberry press-cake extracts. Preliminary investigation in this laboratory has revealed that glycerides are not present in cranberry cuticle lipid, therefore, implicating seeds as the source of these materials in press-cake. As the hydrocarbons of cranberry seed are predominantly of the even carbon number chain type, nonacosane and hentriacontane, as previously reported in press-cake,[3] are probably derived from cuticle lipid. The fatty acid composition of cranberry seeds is very similar to that of the Great Berried Manzanita[9] (*Arctostaphylos glauca*), one of the few other ericaceous fruits examined for seed oil content.

EXPERIMENTAL

Materials

Cranberries used in this study (Howe's variety) were harvested in September 1968 and held at −20°. The juice was pressed from the berries, the pulp air-dried, and the seeds (13·4 per cent moisture) separated by sieving.

Extraction and Purification of Lipids

Cranberry seeds (10 g) were crushed in a mortar under N_2 and immediately extracted with boiling isopropanol, $CHCl_3$–isopropanol and $CHCl_3$, according to Kates and Eberhardt.[11] Combined extracts were concentrated under vacuum and purified by elution through Sephadex with $CHCl_3$–MeOH (1:1) sat. with H_2O. MeOH and H_2O were removed under vacuum by repeated solvent replacement with $CHCl_3$ (yield 23·3 per cent lipid, dry basis).

Column Chromatography

Column chromatography on silicic acid, as described by Rouser *et al.*,[13] yielded three fractions by elution with $CHCl_3$ (neutral lipids, 95·5 per cent), followed by acetone (glycolipids, 3·4 per cent) and MeOH (phospholipids, 1·1 per cent).

Thin-layer Chromatography and Transmission Densitometry

All TLC was performed on 0·25 mm layers of silica gel G. Neutral lipids were developed with $CHCl_3$ while glycolipids and phospholipids were developed with $CHCl_3$–MeOH–H_2O (65:25:4). For transmission densitometry, spots were sprayed with 55% H_2SO_4–1% $K_2Cr_2O_7$ and charred. Spots to be eluted were sprayed with 0·002% Rhodamine 6G, and Liebermann–Burchard spray[22] was used to confirm triterpenoids. Reference standards were co-chromatographed with unknowns.

After charring, developed plates were read on a recording Densicord Electrophoresis Densitometer (Photovolt Corp., New York) running without a filter. A planimeter was used in calculating approximate

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area percentages. The resolution of the diglycerides from the sterols and the monoglycerides from the triterpene acids was insufficient for densitometry. The sample was therefore saponified (see below) and the nonsaponifiables chromatographed and again subjected to densitometry. The sterol and triterpene acid area percentages were then calculated relative to the pentacyclic triterpene alcohol spot. The amount of the mono- and diglycerides were then determined by subtraction of sterol and triterpene acid from the original densitogram.

Two-dimensional TLC of the neutral lipids with $CHCl_3$ followed by hexane–Et_2O–HAc (70:30:1·5) did not yield additional spots.

Saponification of Neutral Lipids and Preparative TLC of Subfractions

Neutral lipids (250 mg) were saponified with 10 ml of 0·5 N methanolic KOH and the non-saponifiables extracted with hexane followed by Et_2O. The pooled concentrate was streaked on a silica gel G wedge plate (0·25–1·0 mm) and developed with $CHCl_3$. Three streaks were detected with Rhodamine 6G (hydrocarbons, pentacyclic triterpene alcohols and sterols), scraped from the plate and eluted with dry Et_2O.

Saponifiables were extracted from the acidified saponification mixture and fatty acids separated from triterpene acids by TLC on a wedge plate using $CHCl_3$–HAc (90:3). Subfractions were prepared as previously described.

Preparation of Methyl Esters and Trimethylsilyl Derivatives

Methylation of neutral lipid fatty acids, glycolipids and phospholipids was carried out with BF_3–MeOH according to the procedures of Morrison and Smith.[16] Trimethylsilyl derivatives of isolated sterol, pentacyclic triterpene alcohol and triterpene acid subfractions were prepared by reaction with bis-trimethylsilylacetamide (1 ml/mg for 12 hr).[15]

Analytical GLC

A Perkin–Elmer 800 gas chromatograph with dual columns and dual flame ionization detectors was employed using the following parameters.

(*a*) Fatty acid methyl esters: 1·8 m × 3 mm ss. column, 8% DEGS-2% phosphoric acid on 80/100 mesh Chromasorb W; 160°, 30 ml/min nitrogen flow.

(*b*) Hydrocarbons: 1·8 m × 3 mm ss. column, 2% OV-1 on 60/80 mesh Gas-Chrom Q; 190°–250° at 4°/min, 30 ml/min nitrogen flow.

(*c*) TMS-sterols and TMS-pentacyclic triterpene alcohols: 2% OV-1 column at 255°, 30 ml/min.

(*d*) TMS-triterpene acids: 2% OV-1 column at 280°, 30 ml/min.

All components were determined by GLC co-injection with authentic standards. Peak areas were calculated by triangulation.

Acknowledgements—The authors are grateful to Dr. P. G. Sammes, Imperial College, London, and Dr. J. McLean, University of Strathclyde, Glasgow, for their generous gifts of triterpene reference samples and to Ocean Spray Cranberries, Inc., South Hanson, Massachusetts, for their contribution of raw material.