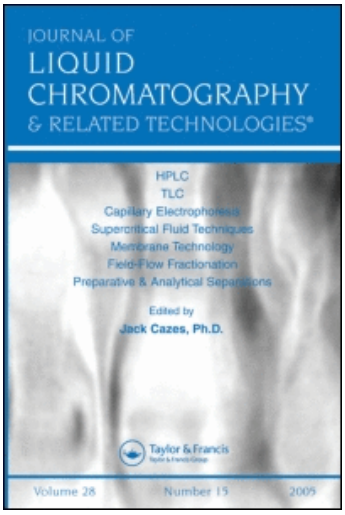

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Simple Liquid Chromatographic Method for Analysis and Isolation of the Unsaturated Components of Anacardic Acid

H. A. Lloyd[a]; C. Denny[a]; G. Krishna[b]
[a] Laboratory of Chemistry, Lung, and Blood Institute National Institutes of Health, Bethesda, Maryland
[b] Laboratory of Chemical Pharmacology National Heart, Lung, and Blood Institute National Institutes of Health, Bethesda, Maryland

To cite this Article Lloyd, H. A. , Denny, C. and Krishna, G.(1980) 'A Simple Liquid Chromatographic Method for Analysis and Isolation of the Unsaturated Components of Anacardic Acid', Journal of Liquid Chromatography & Related Technologies, 3: 10, 1497 — 1504
To link to this Article: DOI: 10.1080/01483918008062791
URL: http://dx.doi.org/10.1080/01483918008062791

JOURNAL OF LIQUID CHROMATOGRAPHY, 3(10), 1497-1504 (1980)

A SIMPLE LIQUID CHROMATOGRAPHIC METHOD FOR ANALYSIS AND ISOLATION OF THE UNSATURATED COMPONENTS OF ANACARDIC ACID

H. A. Lloyd*, C. Denny* and G. Krishna+
*Laboratory of Chemistry and
+Laboratory of Chemical Pharmacology
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, Maryland 20205

ABSTRACT

Good analytical and preparative separation of the saturated, monoene, diene and triene components of anacardic acid have been achieved by reverse phase HPLC on a C_{18} column by isocratic elution with methanol-aqueous acetic acid.

INTRODUCTION

The term "anacardic acid" is used to describe the mixture of 6-n-alkylsalicylic acids which constitute the major part of the extract of cashew nut shells (Anacardium occidentale L.) (1). Its principal components, 6-pentadecyl, 6-(pentadec-8-enyl), 6-(pentadec-8,11-dienyl) and 6-(pentadec-8,11,14-trienyl) salicylic acids are accompanied by small amounts of the C_{13} to C_{17} side chain homologues.

Various methods have been used to determine the composition of anacardic acid, such as gas chromatography of the methyl ethers, argentation thin layer chromatography combined with UV spectrophotometry, and mass spectral analysis of the methyl anacardates (1).

The first complete preparative separation of the olefinic components of anacardic acid was achieved by Paul and Yeddanapalli (2) in 1956 by low-temperature fractional crystallization. The procedure is laborious and gives poor yields of the individual acids. Later, Tyman and his collaborators (3,4) obtained good yields of the pure unsaturated anacardic acids on a preparative scale by argentation-TLC on silica gel plates. This method is also time-consuming as suitable TLC plates of silica gel impregnated with 20% silver nitrate are not available commercially, and moreover only 10-20 mg. of mixture can be separated on one plate in this manner.

Therefore, recently, when we needed the individual pure unsaturated free acids for pharmacological testing, we decided to investigate high pressure liquid chromatography as a potentially simpler and more rapid preparative technique.

HPLC has been applied to the analysis of a number of hydroxybenzoic acids (5,6) but not to the class of phenols or aromatic acids with long alkyl side-chain substituents. Since the anacardic acids had already been separated by argentation-TLC, we first attempted an HPLC separation using a reverse phase column and a mobile phase containing silver nitrate. This method had yielded good results with many alkenes (7), but it was not successful in our hands. We found, however, that a simple, straightforward system using a C_{18} reverse phase column and methanol-aqueous acetic acid gives baseline analytical separation of the constituents of anacardic acid. The method was extended to the separation of the monoene, diene and triene components of more than 200 mg of crude anacardic acid.

MATERIALS AND METHODS

Apparatus

A Waters Associates (Milford, MA) HPLC system consisting of a model 6000A pump, U6K injector, and model 440 dual wavelength UV detector operating at 280 and 313 nm, was used in this study.

The analytical separations were performed on a Partisil-10 ODS-2 column (4.6 x 250 mm) and the preparative runs on a Magnum 9 Partisil-10 ODS-2 column (9.4 x 500 mm). Both columns were obtained from Whatman Inc., Clifton, NJ.

Chemicals

The solvents used were Burdick and Jackson (Muskegan, MI) methanol, reagent grade acetic acid from Baker Chemical Co. (Phillipsburg, NJ), and distilled water purified in a Milli-Q-System from Millipore Corp., Bedford, MA.

The cashew nuts, collected in Florida and in Brazil were treated essentially by Tyman's method (8). The nuts were cooled in liquid nitrogen, cracked, and the shells were separated from the pulp and extracted with ether containing 0.1% of antioxidant 4-methyl-2,6-di-t-butylphenol. The "anacardic acid" was isolated from the total extract according to the lead salt procedure of Backer and Haack (9). The final product, a thick yellowish oil, was examined by TLC and by GC-MS after trimethylsilylation. It contained only anacardic acids and was uncontaminated by purely phenolic compounds.

Procedure

The samples of crude "anacardic acid" for analytical or preparative HPLC were dissolved in the same solvent system used for the separation. The analytical column was eluted with methanol-4% aqueous acetic acid (9:1) at a flow rate of 1 ml/min. In the preparative runs the column was eluted with methanol-4% acetic acid (85:15) at 4 ml/min. and the fractions were collected manually. For each sample, the methanol was removed under reduced pressure, the remaining aqueous solution extracted with chloroform, the extract dried and evaporated, the residue transferred to a vial and dried to constant weight under high vacuum at room temperature. All the residues were reexamined by HPLC and on a LKB-9000 mass spectrometer by direct probe MS and by GC-MS as trimethylsilyl derivatives. The derivatives were prepared by heating the compounds in acetonitrile with bis-(trimethylsilyl)-trifluoroacetamide for 10 min. at 140°C.

RESULTS AND DISCUSSION

Of the various columns and mobile phase combinations which we investigated, the best resolution was achieved with a Partisil-10 column (Whatman Inc.) and isocratic elution with methanol-water-acetic acid. On this column, the saturated and the mono-, di-, and triunsaturated 6-n-C_{15}-alkylsalicylic acids separated even with pure methanol; addition of 10% water gave baseline resolution and further addition of 2-4% acetic acid to the water improved both resolution and peak shapes.

A typical elution pattern with a mixture of methanol and 4% acetic acid in water in a ratio of 9:1 is illustrated in Figure 1. Shoulders on the main peaks and other small peaks between the larger ones reveal the presence of additional com-

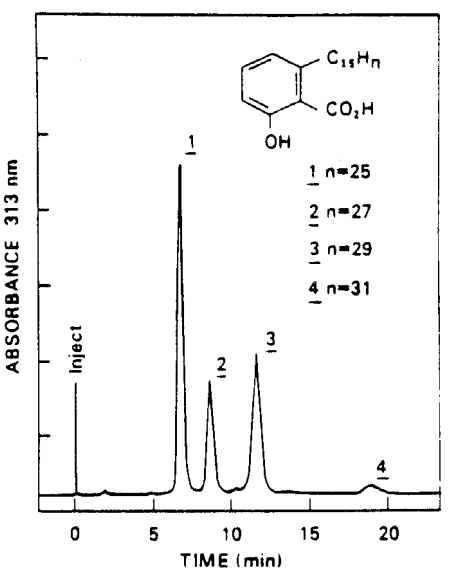

FIGURE 1. HPLC separation of anacardic acids (Florida nuts). Column : Partisil-10 ODS-2, Mobile phase : methanol-4% aqueous acetic acid (9:1). Flow rate : 1 ml/min. UV detector, 313 nm, 0.1 AUFS. Load 2 µg in 2 µl solvent. 1 : triene; 2 : diene; 3 : monoene; 4 : saturated compound.

ponents in the mixture. In order to retain optimum resolution on the larger column with much larger sample loads, the proportion of aqueous acetic acid was increased to 15%. Figure 2 shows the separation of 240 mg of crude "anacardic acid" run on a Magnum 9 Partisil ODS-2 column. Analytical runs were monitored at 280 nm as well as at 313 nm, near the UV maximum of the anacardic acids (308 nm). With preparative scale samples, our detector was overloaded at 313 nm but it still gave a linear response at 280 nm where the UV absorption of the anacardic acids is much smaller.

The fractions were collected as indicated on Figure 2 and analyzed through GC-MS of their trimethylsilylation products. The main components, C_{15} triene, diene and monoene, eluted in the

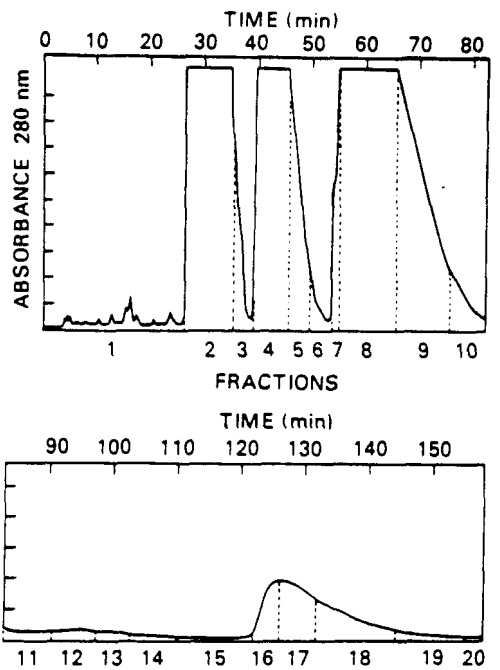

FIGURE 2. Preparative HPLC separation of anacardic acids (Florida nuts). Column : M-9 Partisil-10 ODS-2. Mobile phase: methanol-4% aqueous acetic acid (85:15). Flow rate : 6 ml/min. UV detector, 280 nm, 2.0 AUFS. Load : 240 mg in 0.6 ml solvent.

expected order of polarity, so did the traces of C_{13}, C_{14}, C_{16} and C_{17} homologues. In subsequent runs only the olefinic C_{15} compounds were collected and the operational time was shortened by washing the column with methanol after elution of the monoene. The weight and composition of material isolated from the various fractions are given in Table 1; over 96% of the material injected was recovered.

TABLE 1. Composition of the Preparative HPLC Fractions

Fract. No.	Weight mg	Composition
1.	3.2	mixture of 6-n-C_{15}-resorcinols
2.	86.1	pure C_{15}triene
3.	3.8	C_{15}triene; tr. C_{13}monoene
4.	34.6	pure C_{15}diene
5.	6.4	pure C_{15}diene
6.	1.3	C_{15}diene; tr. C_{14}monoene
7.	2.5	99% C_{17}triene; tr.C_{13}sat.,tr.C_{15}diene; tr.C_{15}monoene
8.	48.5	pure C_{15}monoene
9.	19.6	pure C_{15}monoene
10.	2.3	C_{15}monoene; tr. C_{17}diene
11.	1.6	ca. 1/3 C_{15}monoene; 2/3 C_{17}diene
12.	1.4	C_{17}diene; tr.C_{14}sat.
13.	1.8	ca. 3/4 C_{17}diene; 1/4 C_{16}monoene; tr.C_{14}sat.
14.	1.6	fatty acids
15.	1.2	fatty acids
16.	2.7	pure C_{15}sat.
17.	3.7	C_{15}sat.; tr.C_{17}monoene
18.	3.4	ca. 2/3 C_{15}sat.; 1/3 C_{17}monoene
19.	1.5	ca. 1/3 C_{15}sat.; 2/3 C_{17}monoene
20.	4.1	non acidic or phenolic material
Total	231.3	

The amounts and proportions of olefinic and saturated C_{15} constituents taken from the table are as follows: triene, 89.9 mg.(42.4% of total C_{15}); diene 42.3 mg. (19.9%); monoene, 70.9 mg. (33.4%); saturated, 9.1 mg. (4.3%). These ratios vary slightly from those reported by Tyman and Jacobs (4) for nuts of a different origin, but the overall degree of unsaturation of the total C_{15} anacardic acids averages out to two double bonds in agreement with the findings of Tyman and of previous researchers.

Our primary concern in this investigation was to prepare sufficient amounts of the pure individual olefinic acids for pharmacological and toxicological testing. This same HPLC separation can also be applied to a rapid quantitation of the anacardic acid components in cashew nutshells of different origins and the procedure appears to be much simpler than other methods for quantitative analysis of anacardic acid.

Currently we are studying the role of the various anacardic acid constituents as specific inhibitors of prostacyclin receptor in human blood platelets. In another investigation we have found that the triene anacardic acid is a potent molluscicide against B. glabrata, a snail which normally harbors schistosomes; thus, it may be possible to use anacardic acid or even the crude cashew nutshell liquid to control the population of fresh water snails and the spreading of schistosomiasis. The results of these studies will be published elsewhere.

ACKNOWLEDGEMENT

We are grateful to Dr. Julia Morton, University of Miami, Coral Gables, FL and Dr. Wilson Yasaka, University of Sao Paulo, Brazil, for supplying the cashew nuts used in this study.

REFERENCES

1. Tyman, J. H. and Kiong, L. S. Longchain Phenols. XI. Composition of Natural Cashew Nutshell Liquid (Anacardium occidentale) from Various Sources. Lipids 13, 525 (1978) and references therein.

2. Paul, V. J. and Yeddanapalli, L. M. On the Olefinic Nature of Anacardic Acid from Indian Cashew Nut Shell Liquid. J. Am. Chem. Soc., 78, 5675 (1956).

3. Tyman, J. H. P. and Morris, L. J. The Composition of Cashew Nut Shell Liquid (CNSL) and the Detection of a Novel Phenolic Ingredient. J. Chromatog., 27, 287 (1967).

4. Tyman, J. H. P. and Jacobs, N. The Composition of the Unsaturated Phenolic Components of Anacardic Acid. J. Chromatog., 54, 83 (1971).

5. Murphy, J. B. and Stutte, C. A. Analysis for Substituted Benzoic and Cinnamic Acids using High Pressure Liquid Chromatography. Anal. Biochem., 86, 220 (1978).

6. Scriven, F. M., Day, W. R. and Willis, R. B. H. Analysis of Hydrobenzoic Acids by High Pressure Liquid Chromatography. J. Liquid Chromatog. 2, 125 (1979).

7. Vonach, B. and Schomburg, G. High-Performance Liquid Chromatography with Ag+ Complexation in the Mobile Phase. J. Chromatog., 149, 417 (1978).

8. Tyman, J. H. P. Long-chain Pehnols. Part III. Identification of the Components of a Novel Phenolic Fraction in Anacardium occidentale. J. Chem. Soc., Perkin Trans. I, 1639 (1973).

9. Backer, H. J. and Haack, N. H. Composants du Latex de l' Anacardium occidentale Linn. Rec. Trav. Chim., 60, 661 (1941).