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Note

Use of silver nitrate impregnated silica cartridges in the separation of kahweol and cafestol esters by preparative liquid chromatography

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Cafestol and kahweol are diterpenoid coffee constituents present in the light petroleum (boiling range 60–70°C) extract of green coffee beans in the form of esters of fatty acids[1,2]. These compounds are potent inducers of the activity of glutathione (GSH) S-transferase which is a major detoxifying enzyme system that catalyses the binding of a variety of electrophiles, including reactive forms of chemical carcinogens to GSH[3]. The palmitates of cafestol and kahweol were found to inhibit 7,12-dimethylbenz[*a*]anthracene-induced mammary tumor formation in the rat[4].

Cafestol and kahweol are diterpene alcohols, differing only in a $\Delta 1$ double bond present in kahweol (Fig. 1). Separation of the parent alcohols and of their esters was difficult because of the similarity in structure of these compounds. It was necessary to devise a method of separation so that sufficient quantities of pure cafestol palmitate and kahweol palmitate could be prepared.

Analytical samples of kahweol palmitate and cafestol palmitate have been separated into their pure components by silver nitrate impregnated silica gel thin-layer plates[3]. Extending this technique we have developed preparative liquid chromatography (LC) silica cartridges which were impregnated with 10% silver nitrate to achieve complete separation of gram quantities of the diterpene esters without extensive recycling.

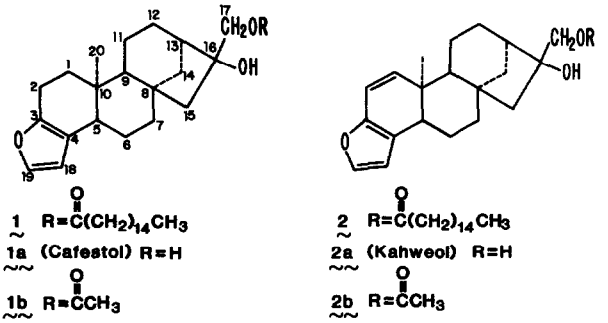

Fig. 1. Cafestol, kahweol and their acetates and palmitates.

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EXPERIMENTAL

Preparation of 10% silver nitrate–silica

A 20.0-g amount of silver nitrate (99.9 + % pure, Alfa Products) was dissolved in 40 ml distilled water. This 50% silver nitrate solution was added slowly to 180 g Kieselgel 60 silica gel (40–63 μm particle size, Merck reagent) and hand mixed during this addition. The silver nitrate–silica mixture was then placed in a plastic container and mixed for 0.5 h using a PTFE stirrer blade, a heavy duty glass stirrer shaft and a Talboy T-Line laboratory stirrer. The silver nitrate–silica mixture was then spread in a thin layer on stainless-steel trays and dried in an oven maintained at 105–120°C for 17 min to remove the water.

Preparation of 10% silver nitrate–silica cartridges

The frit on one end of a Waters PrepPAK 500 silica cartridge (Waters Assoc.) was removed and the silica contents emptied. The empty cartridge was then dry packed with the dried 10% silver nitrate–silica using a Thermolyne Max-Mix vibrator to remove any trapped air. A suction flask with a large rubber adapter and a water aspirator were used during the dry packing to give a hard solid column of packing material. When completely filled, the end frit was pressed in place. The silver nitrate–silica content of a packed cartridge was approximately 480 g.

Preparative separation of cafestol and kahweol esters

The crude mixture of cafestol and kahweol esters was prepared by the reaction of cafestol–kahweol mixture with the appropriate acid chloride. Preliminary separation of the esters from other coffee extracts was carried out with a Waters preparative LC system 500 chromatograph using 2 PrepPAK silica cartridges; mobile phase, hexanes–ethyl acetate (2:1, v/v); flow-rate, 200 ml/min.

The cafestol–kahweol ester mixture was then separated into their pure components using two 10% silver nitrate impregnated silica cartridges; mobile phase, hexanes–ethyl acetate (2:1, v/v); flow-rate, 200 ml/min.

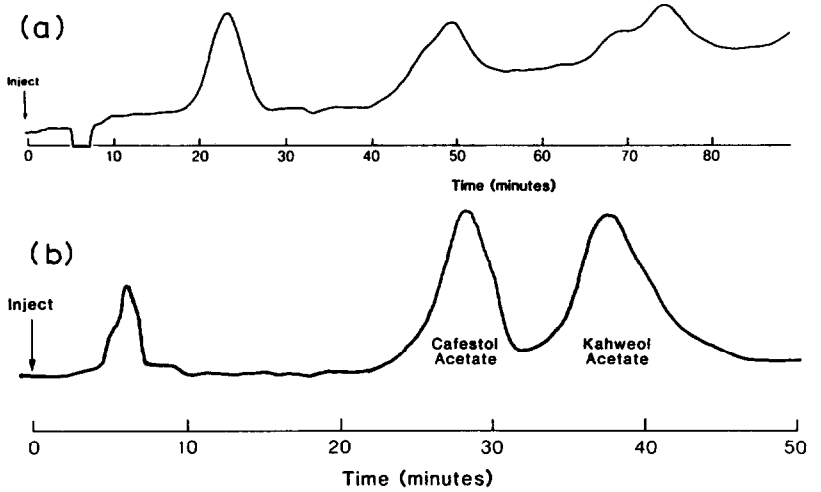

Fig. 2. Preparative LC chromatograms of normal phase separation of a mixture of cafestol and kahweol acetates [mobile phase, hjexanes–ethyl acetate (2:1); flow-rate, 200 ml/min] by (A) recycling mode on two PrepPAK 500/silica cartridges (detection, 100 relative response) and (B) by single pass on two 10% silver nitrate–silica cartridges (detection, 50 relative response).

Collected fractions were analyzed by thin-layer chromatography (TLC), using silver nitrate impregnated silica gel TLC plates [mobile phase, hexanes–ethyl acetate (1:2, v/v); sprayed with 0.1% vanillin–sulfuric acid reagent; cafestol esters gave an orange spot, kahweol esters gave a purple spot].

RESULTS AND DISCUSSION

Complete separation of cafestol and kahweol esters was successful by means of two 10% silver nitrate impregnated silica cartridges. Fig. 2 shows the separation of a mixture of cafestol and kahweol acetates in a single pass. In contrast, repeated recycling of a mixture of cafestol and kahweol acetates through two regular silica cartridges (PrepPAK 500/silica, Waters Assoc.) failed to separate the acetates into their individual components.

Chromatographic data shown in Tables I and II indicate the advantages of silver nitrate impregnated silica cartridges over that of the regular silica cartridges for the separation of cafestol and kahweol acetates.

The palmitates and other esters of cafestol and kahweol were separated similarly by silver nitrate impregnated silica cartridges in large quantities.

TABLE I

SEPARATION OF CAFESTOL ACETATE AND KAHWEOL ACETATE ON TWO 10% SILVER NITRATE IMPREGNATED SILICA CARTRIDGES

Mobile phase, hexanes–ethyl acetate (2:1).

	k'	N	α	R_s
Cafestol acetate	4.72	349	140	1.26
Kahweol acetate	6.60	296		

TABLE II

SEPARATION OF A MIXTURE OF CAFESTOL ACETATE AND KAHWEOL ACETATE ON TWO REGULAR SILICA CARTRIDGES

Mobile phase, hexanes–ethyl acetate (2:1).

	k'	N	α	R_s
Mixture	3.72	159	0	0

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REFERENCES

1 C. Djerassi, E. Wilfred, L. Visco and A. J. Lemin, *J. Org. Chem.*, 18 (1953) 1449–1460.
2 H. P. Kaufmann and A. K. Sen Gupta, *Chem Ber.*, 96 (1963) 2489–2498.
3 L. K. T. Lam, V. L. Sparnins and L. W. Wattenberg, *Cancer Res.*, 42 (1982) 1193–1198.
4 L. W. Wattenberg and L. K. T. Lam, *Banbury Rep.*, 17 (1984) 137–145.