Journal of Chromatography, 538 (1991) 227–229
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 721

Note

Separation of quassinoids from *Ailanthus altissima* by high-speed counter-current chromatography

M. JAZIRI*
Laboratory of Plant Morphology, Free University of Brussels, 1850 Ch. de Wavre, B-1160 Brussels (Belgium)
and
B. DIALLO and M. VANHAELEN
Laboratory of Pharmacognosy, Campus Plaine, Bld. Triomphe, B-1050 Brussels (Belgium)

Quassinoids are bitter constituents found in most *Simaroubaceae* sp. and show interesting biological activities. In order to obtain standards of these compounds for the survey of metabolite production in *Ailanthus altissima* cell cultures [1,2], isolation of quassinoids from the root-bark of the plant was undertaken. The separation and purifiation of quassinoids have been usually acieved using combined chromatographic methods [column chromatography on silica gel or on reversed-phase silica gel, preparative tin-layer chromatography (TLC)]. High-speed counter-current chromatography (HSCCC) has not previously been used for the separation of this group of natural products.

EXPERIMENTAL

All chemicals were of analytical-reagent grade.

Apparatus

HSCCC was performed using an Ito multi-layer coil separator–extractor [3] (P.C., Potomac, MD, U.S.A.) equipped with a 2.6 mm I.D. column. An LDC Milton Roy (Riviera Beach, FL, U.S.A.) minipump was used to pump the solvents through the column. The rotational speed was 800 rpm. A manual sample injection valve (Lobar Column Accessories, Merck) equipped with a 10-ml loop was used to introduce the sample into the column. Fractions were collected using a LKB Ultrorac 7000 collector.

Preparation of sample

Powder of dried root-bark of *A. altissima* (350 g) was extracted with methanol and the methanolic extract was further suspended in light petroleum. The light petroleum-insoluble residue was dissolved in a small volume of methanol, absorbed on

0021-9673/91/$03.50

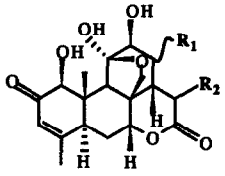
cellulose and chromatographed on a silica gel column. Elution was achieved with chloroform containing increasing amounts of methanol. The fractions eluted with chloroform–methanol (95:5) were evaporated under reduced pressure, chromatographed on a reversed-phase silica gel column and eluted with water–acetonitrile (80:20). Fractions containing ailanthone and a mixture of related quassinoids were evaporated and the residue (80 mg) was used for the HSCCC separation.

Separation procedure

A two-phase solvent system was prepared by equilibrating chloroform–methanol–water (5:6:4). After separation, the two phases were degassed in an ultrasonic bath. The upper phase, used as the stationary phase, was pumped into the column at 6 ml/min. The sample (80 mg) was dissolved in 6 ml of each phase and introduced through the injection port. The lower phase, used as the mobile phase, was then pumped into the column at 4 ml/min. The separation was performed at room temperature and 30 fractions of 15 ml were collected.

TABLE I

HSCCC OF THE QUASSINOIDS ISOLATED FROM A PREPURIFIED EXTRACT (80 mg) OF *A. ALTISSIMA* ROOT-BARK



	R_1	R_2
1 Chapparinone	····· CH_3	H, H
2 Ailanthone	= CH_2	H, H
3 13,18-Dehydroglaucarubinone	= CH_2	OOC - C(OH)(CH_3) - C_2H_5, H
4 13,18-Dehydroglaucarubolone	= CH_2	OH

Fractions	R_F (TLC)	Constituent	Amount (mg)
1–3	–	–	
4	0.56	1	18
5–6	0.50–0.42	2+3	10
7–8	0.42	3	24
9–25	–	X[a]	17
26–30	0.25	4	10
		Total:	79

[a] X corresponds to a mixture of three compounds that were not identified; on the basis of co-chromatography with an authentic sample, the presence of chapparin in this mixture is likely.

Fractionation monitoring

The purity of fractions was checked by TLC on silica gel using chloroform–methanol (98:2) as the mobile phase; detection was achieved under UV radiation at 254 nm and also by spraying a 3% solution of sulphuric acid in methanol followed by heating for 5 min at 115°C.

RESULTS AND DISCUSSION

The solvent system was chosen on the basis of the partition coefficient of the quassinoids in the two solvent phases and TLC. The order of elution from the column corresponded to decreasing R_F values of the different compounds after TLC (Table I). Quassinoids 1, 3 and 4 were obtained in pure form and used directly without any further purification for structure determination (UV, mass and ^{1}H NMR spectrometry). No loss of constituents occurred during the separation, which was achieved within 2 h.

REFERENCES

1 M. Jaziri, J. Homès and M. Vanhaelen, *Phytochemistry*, 26 (1987) 999.
2 M. Jaziri and M. Vanhaelen, *Meded. Fac. Landbouwwet. Rijksuniv. Gent*, 4 (1987) 52.
3 Y. Ito, *Anal. Chem.*, 17 (1986) 65.
4 D. G. Martin, C. Biles and R. E. Peltonen, *Am. Lab.*, Oct. (1986) 21.