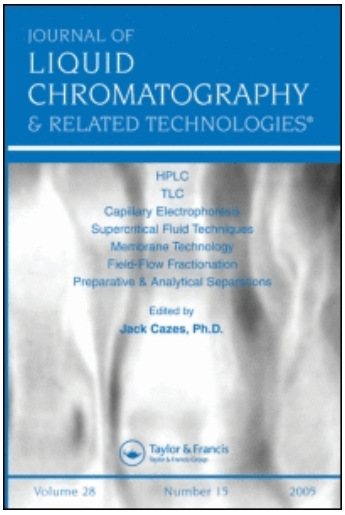

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Application of High Speed Countercurrent Chromatography/Thermospray Mass Spectrometry for the Analysis of Bio-Active Triterpenoic Acids from Boswellia Carterh

Y. -W. Lee[a]; T. W. Pack[a]; R. D. Voyksner[a]; Q. C. Fang[b]; Y. Ito[c]
[a] Chemistry and Life Sciences Research Triangle Institute, Research Triangle Park, North Carolina [b] Department of Natural Products Chemistry, Institute of Materia Medica Chinese Academy of Medical Sciences, Beijing, China [c] Laboratory of Chemistry, National Heart, Lung and Blood Institute, Bethesda, Maryland

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APPLICATION OF HIGH SPEED COUNTERCURRENT CHROMATOGRAPHY/ THERMOSPRAY MASS SPECTROMETRY FOR THE ANALYSIS OF BIO-ACTIVE TRITERPENOIC ACIDS FROM BOSWELLIA CARTERII

Y.-W. LEE[1], T. W. PACK[1], R. D. VOYKSNER[1],
Q. C. FANG[2], AND Y. ITO[3]
[1]*Chemistry and Life Sciences*
Research Triangle Institute
P. O. Box 12194
Research Triangle Park, North Carolina 27709
[2]*Department of Natural Products Chemistry*
Institute of Materia Medica
Chinese Academy of Medical Sciences
Beijing, China
[3]*Laboratory of Chemistry*
National Heart, Lung and Blood Institute
Bethesda, Maryland 20892

ABSTRACT

The versatility and high resolving power of countercurrent chromatography has been demonstrated with a newly developed analytical high speed planet centrifuge system (HSCCC). Interfacing countercurrent chromatography with mass spectrometry (MS) provides a new analytical methodology which integrates the advantages of countercurrent chromatography with the low detection limit and identification capability of mass spectrometry. The capability of thermospray HSCCC/MS has been successfully demonstrated in the identification of plant alkaloids and lignans. In this paper, the technique proved useful in identifying and validating the bio-active and structural closely related triterpenoid carboxylic acids from a crude extract of Boswellia carterii (Burseraceae). Thermospray HSCCC/MS can become a useful and complementary method to thermospray HPLC/MS for the analysis of nonvolatile or thermally unstable molecules.

INTRODUCTION

The separation of multicomponent mixture by differential partition between two immiscible solvents has long been recognized. In spite of the limitations of traditional countercurrent distribution methods which prevailed in the late 1950's and early 1960's, its remarkable ability to resolve mixtures was clearly demonstrated in the fractionation of commercial insulin (molecular weight 6000) into two subfractions which differ only by an amide group [1].

In recent years, significant improvements have been made to enhance the performance and efficiency of countercurrent methods [2]. The newly developed high speed CCC (HSCCC) utilizes a particular combination of coil orientation and planetary motion which produces a unique hydrodynamic phenomenon of a unilateral phase distribution of two immiscible solvents in a coiled column. This hydrodynamic behavior can be effectively applied to perform a variety of countercurrent chromatographies, including true countercurrent [3] and foam countercurrent methods [4]. Recently, development of high speed CCC (2000 rpm) has provided the CCC system with the higher efficiency required for analytical work. The separation achieved is approaching HPLC in terms of resolution and speed [5]. We have successfully applied the HSCCC system in the separation of plant alkaloids, plant indole hormones, herbicides and bioactive lignans. One distinct advantage of HSCCC is that no complications arise from solid supports, such as adsorptive sample loss, deactivation and contamination.

Because of the two phase solvent system commonly employed in HSCCC often consists of organic phase and aqueous phase, it is well suited to thermospray mass spectrometry (HSCCC/MS). Mass spectrometry (MS) is generally regarded as a versatile and widely applicable detection technique. The advantage of MS detection rather than more conventional detectors such as UV for HSCCC is its independance of a UV chromophore and its ability to identify peaks.

Integration of the HSCCC to the mass spectrometer allows spectra to be acquired continuously. In addition, individual chromatographic peak need not be pure to acquire mass spectra for identification. One technique that exploits this advantage is to use reconstructed single-ion plots in which all of the ions of a given m/z value are plotted against retention time. If the intensity of a reconstructed single-ion plot for a given m/z does not rise and fall in accordance with a peak in the total ion current chromatogram (TIC), then other components are present. Also the on-line HSCCC/MS technique provides data on trace components that are not easily obtained in the off-line techniques due to limited resolution.

Interfacing countercurrent chromatography with thermospray [6] mass spectrometry appears incompatible due primarily to the back pressure(500-1000 psi) generated by the heated thermospray vaporizor, which exceeds the pressure limitation of the countercurrent system. Until rcently, development of HSCCC system provided not only the efficiency but also the pressure tolerance required for the HSCCC/MS coupling. In addition, the high percentage aqueous condition with a volatile buffer (e.g., ammonium acetate) used in HSCCC provides the best thermospray MS sensitivity [7] and the minimum losses in chromatographic resolution typically observed in HPLC when the mobile phase is switched to higher aqueous percentages.

The HSCCC/MS technique has been successfully applied in the analysis of bio-active plant alkaloids [8] and lignans [9]. This paper describes the application of thermospray HSCCC/MS in the identification of bio-active triterpenoic acids from Boswellia carterii, a traditional Chinese herbal medicine for treatment of rheumatoid arthritis and cancers.

EXPERIMENTAL SECTION

Reagents and Materials

Ethanol and n-hexane used for preparation of the two phase solvent systems were glass distilled chromatographic grade purchased from Burdick and

Jackson Laboratories, Inc., Muskegon, MI. Experiments were performed with a two phase solvent system composed of n-hexane, ethanol, and water with a volume ratio of 6:5:1. The two phase solvent system was prepared by thoroughly equilibrating the solvent mixture in a separatory funnel at room temperature followed by filtration and degassing with a 5 μm filter.

Analytical High Speed CCC:

A newly developed analytical high speed planet centrifuge equipped with a multilayer coil column of 0.85 mm i.d PTFE tubing was employed. The system is capable of revolution at 2000 rpm with a 5 cm radius [3]. A Waters 6000A HPLC pump (Waters Associates, Milford, MA) was used for the mobile phase. UV detection was achieved with an ISCO Model 1840 (Lincoln, Nebraska) Variable Wavelength UV-Vis absorbance detector. The column was first filled with the stationary phase (upper phase); then the mobile phase (lower phase) was pumped at 0.7 mL/min while the column was spun at 1500 rpm. The sample solution was injected when the HSCCC system had reached equilibrium as indicated by a clean mobile phase eluted.

Thermospray HSCCC/MS

The effluent from the HSCCC (0.8 mL/min) was introduced into a Waters 6000A pump through a zero dead volume tee fitted with a reservoir. The Waters pump was necessary to achieve the solvent pressure required for thermospray. Also, since the HSCCC showed flow rate variations, the thermospray Waters pump was operated at 0.7 mL/min with the reservoir providing either extra solvent or venting excess solvent from the HSCCC system. The effluent from the Waters pump was mixed coaxially with 0.3 M ammonium acetate added at 0.3 mL/min to provide the volatile buffer for ion evaporation ionization [10]. This combined effluent (total of 1 mL/min) first passed through a UV detector (254 nm) then into the thermospray interface. At lower HSCCC flow rates (0.3-0.6 mL/min) the pressure drop across the thermospray vaporizer was sufficiently low to permit direct coupling of the HSCCC effluent to the thermospray inter-

face without the use of the booster HPLC pump. Post column addition of buffer and UV detection of the HSCCC effluent was maintained as described above.

The thermospray interface (Vestec, Houston, TX) was installed on a Finnigan 4500 quadrupole mass spectrometer. The interface included a temperature controller and read-out. The temperature zones monitored were the vaporizer, source and aerosol (just past the ion exit cone). Electrical cartridge heaters were used in the source and the vaporizer was directly heated. The thermospray interface was operated at a source temperature of 250°C and a vaporizer temperature to maximize the HPLC solvent clusters (about 170°C). The solvent cluster has been shown to co-maximize with the analyte being analyzed.7 This interface did not require any splitting of the HSCCC effluent. The large volume of solvent was pumped out of the source with a liquid nitrogen cold trap prior to the mechanical rough pump. Both negative and positive ion detection using ion evaporation ionization and chemical ionization (CI) were employed for the analysis. The filament was operated at 1000 V with a 0.15 mA emission current. The mass calibration of the quadrupole was verified with polypropylene glycol (AMW 1000).

RESULTS AND DISCUSSION

The HSCCC/UV chromatogram for the crude triterpenoic acids from Boswellia carterii shows the three distinct peaks (Figure 1). Thermospray HSCCC/MS was used to aid in their identifications and further identified the minor components present in the extract. The HSCCC/MS selected ion chromatograms (Figure 2) was helpful in detecting seven triterpenoic acids from Boswellia carterii. The tentative identity of each triterpenoic acid was assigned based on the thermospray positive and negative ion spectra (Table 1). The major HSCCC/UV signals were identified as BC-2, BC-3 and the unresolved mixtures of BC-4 and BC-5 using HSCCC/MS. The use of negative ion MS detection enabled the differentiation of BC-4 from BC-5 (Table 1) as well as enabled the detec-

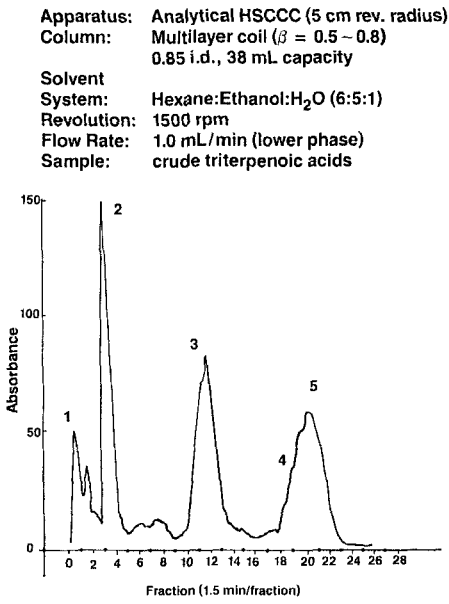


Figure 1: Analytical High Speed Countercurrent Chromatogram

tion of a trace component--2 daltons lower than BC-4 and BC-5. The addition of a double bond to BC-4 and BC-5 appears to be resulted from dehydration of the C-11 hydroxyl group. This was confirmed by NMR analysis. The similarity of these four components accounts for their close retention times by HSCCC, resulting in one broad peak using UV detection; and the relatively long retention accounted for the wide peak width for the components at the end of the ion chromatogram. Additionally, the small early eluting peak (BC-1) was detected by CCC/MS and identified as 11-keto derivative of BC-5 (Table 1).

The thermospray spectra of the triterpenoic acids were relatively simple (Table 1). They exhibited $[M + NH_4]^+$ and $[M + H]^+$ ion and fragment ion from

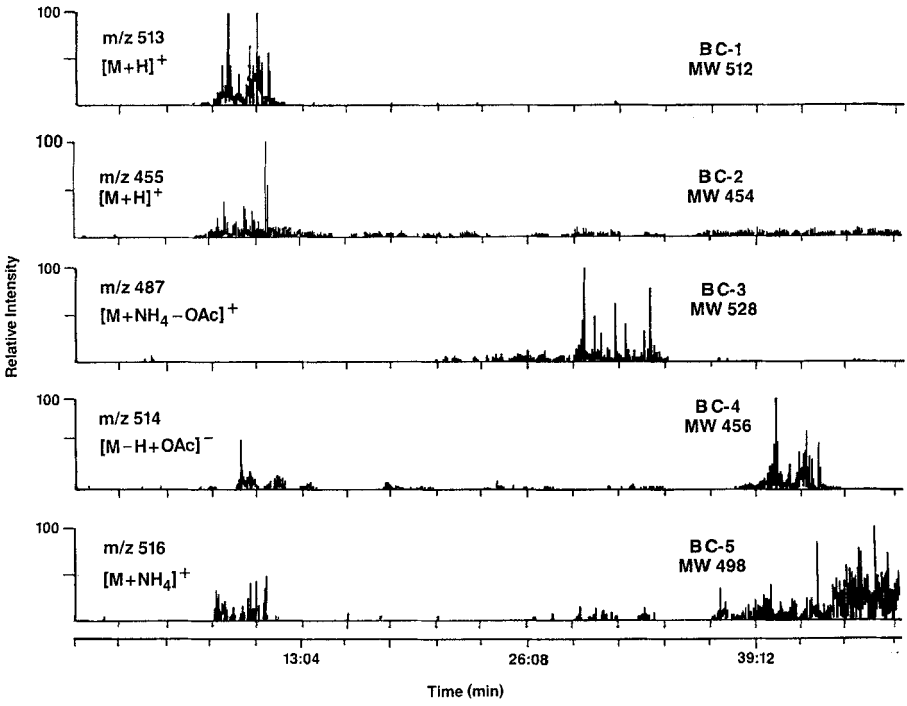
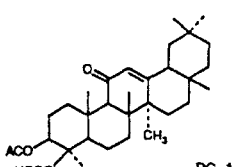
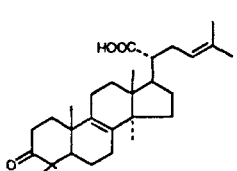
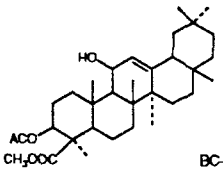
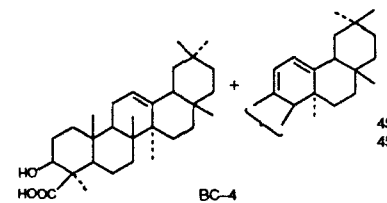
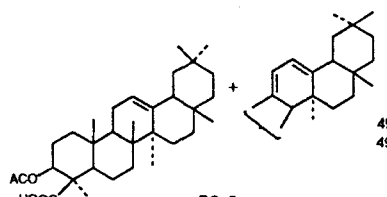


Figure 2: CCC/MS Selected Ion Chromatograms

losses of H_2O, CO_2 and acetate (OAc). The negative ion spectra exhibited $[M + OAc]^-$ and $[M - H]^-$ ions with no significant fragmentation. While negative ion detection sensitivities were significantly lower than positive ion detection (factor of 10 lower), the acquisition of negative ion spectra for BC-4 and BC-5 proved vital for this identification. The positive ion spectra of BC-4 and BC-5 were similarly due to clustering of BC-4 with acetate and BC-5 with $[NH_4]^+$, while the negative ion spectra resulted in two ions for confirming molecular weight (e.g., $[M -H]^-$ and $[M + OAc]^-$).

TABLE 1
TRITERPENOIC ACIDS FROM BOSWELLIA CARTERII DETECTED
IN THERMOSPRAY CCC/MS ALONG WITH POSITIVE AND NEGATIVE ION IDENTIFICATION

Peak No.	Triterpenoic Acids	M.W.	Thermospray Spectra					
			(+) ion			(-) ion		
			m/z	% RI (1)	Identity	m/z	% RI	Identity
1	 BC–1	512	513 453 409	100 40 43	$[M + H]^+$ $[M + H - HOAc]^+$ $[M + H - HOAc - CO_2]^+$	571 511	8 100	$[M + OAc]^-$ $[M - H]^-$
2	 BC–2	454	472 455 437	68 100 12	$[M + NH_4]^+$ $[M + H]^+$ $[M + H - H_2O]^+$	513 453	14 100	$[M + OAc]^-$ $[M - H]^-$

3	 BC–3	528	528	5	$[M + NH_4 - H_2O]^+$	587	3	$[M + OAc]^-$
			498	48	$[M + NH_4 - H_2O - CH_2O]^+$	527	100	$[M - H]^-$
			487	42	$[M + NH_4 - OAc]^+$	455	16	$[M - H - CH_2CH_2COO]^-$
			454	18	$[498 - CO_2]^+$			
			438	100	$[498 - HOAc]^+$			
4	 BC–4	456	516	28	$[M + H + OAc]^+$	515	5	$[M + OAc]^-$
		454	514	78	(1)	513	13	(1)
			456	38	$[M + NH_4 - H_2O]^+$	497	40	$[M + OAc - H_2O]^-$
			454	100	(1)	495	100	(1)
						455	31	$[M - H]^-$
						453	27	(+)
5	 BC–5	498	516	54	$[M + NH_4]^+$	557	22	$[M + OAc]^-$
		496	514	43	(1)	555	17	(1)
			456	100	$[M + NH_4 - HOAc]^+$	497	100	$[M - H]^-$
			454	80	(1)	495	82	(1)
			438	16	$[M + NH_4 - HOAc - H_2O]^+$			
			436	13	(1)			

CONCLUSION

The coupling of HSCCC to MS is in its early stages and new refinements are continually being made. Thick-walled tubing and connections that withstand the high back pressure (roughly 600 psi at 1.0 ml/min) of thermospray MS will make HSCCC/MS more convenient and easy to use. An analytical CCC system capable of operating at 4,000 rpm has been developed in the Laboratory of Technical Development at the U.S. National Institutes of Health which should raise the separation capabilities of the technique.

REFERENCES

1. Ham's, J. I.; Sanger, F.; and Naughton, M. A. Arch. Biochem. Biophys., 65, 427, 1956.
2. Ito, Y. CRC Crit. Rev. Anal. Chem., 17, 65, 1986.
3. Lee, Y. W.; Cook, C. E.; and Ito, Y. J. Liq. Chromatogr., 11, 37, 1988.
4. Ito, Y. J. Liq. Chromatogr., 8, 2131, 1985.
5. Lee, Y. W.; Ito, Y.; Fang, Q. C.; and Cook, C. E. J. Liq. Chromatogr., 11, 75, 1988.
6. Blakely, C. R. and Vestal, M. L. Anal. Chem., 55, 700, 1983.
7. Voyksner, R. D. and Haney, C. A. Anal. Chem., 57, 991, 1985.
8. Lee, Y. W.; Voyksner, R. D.; Fang, Q. C.; Cook, C. E.; and Ito, Y. J. Liq. Chromatogr., 11, 153, 1988.
9. Lee, Y. W.; Voyksner, R. D.; Fang, Q. C.; Pack, T. W.; Ito, Y.; and Cook, C. E. Anal. Chem., submitted 1988.
10. Voyksner, R. D.; Bursey, J. T.; and Pellizzari, E. D. Anal. Chem., 56, 1507, 1984.