Journal of Natural Products
Vol. 49, No. 6, pp. 1133-1137, Nov-Dec 1986

USE OF OVERPRESSURE LAYER CHROMATOGRAPHY (OPLC) FOR THE SEPARATION OF NATURAL PRODUCTS WITH ANTINEOPLASTIC ACTIVITY

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Although the first paper on overpressure layer chromatography (oplc) was published in 1979 (1), and oplc instrumentation has now been available for several years, this planar separation technique has received scant attention in the United States to date. In oplc, the vapor phase is eliminated to leave the sorbent layer completely covered with an elastic membrane under external pressure. When forced by a pump, the mobile phase migrates through the sorbent layer due to the cushion system at overpressure (1-6). The theoretical basis for oplc has appeared in the literature (4-6), and, in addition to its analytical use, the method can be used preparatively (7,8). In comparison with capillary-controlled systems, such as tlc, higher efficiencies, increased solute-loading capacity, and reduced separation times can be achieved by oplc (5,6). Oplc has been shown to provide equivalent resolution to hplc in the analytical separation of some aflatoxins, and several practical advantages of oplc over hplc have been enumerated (9).

In this communication, we wish to report the application of oplc to the analytical separation of some antineoplastic simaroubolides, podophyllotoxin-type lignans, and *Camptotheca* alkaloids. The samples used were either isolated from plant extracts by bioactivity-guided fractionation in this laboratory or were donated. The resolution obtained within each compound class by conventional analytical tlc was progressively improved by oplc using regular (oplc-tlc) and high-performance (oplc-hptlc) tlc plates. We also have used oplc as an online preparative technique for the rapid isolation of impure simaroubolides and lignans.

Comparative tlc, oplc-tlc, and oplc-hptlc separations for the mixtures of simaroubolides, podophyllotoxin-type lignans, and *Camptotheca* alkaloids are shown in Figures 1-3, respectively. Sorbent layers were monitored by densitometry at the conclusion of each separation. Optimum solvent systems were developed by the "PRISMA" technique (10,11) for the tlc separations of the simaroubolide and lignan mixtures. Slight modifications of these solvent compositions were necessary to ensure the optimization of the respective oplc separations. The *Camptotheca* alkaloids investigated were more easily resolvable than the other two mixtures used, and, therefore, the same binary solvent mixture was employed for both tlc and oplc separations. For this reason, it was not considered necessary to use continuous development (overrunning) for the alkaloid oplc separations, as was the case for the simaroubolides and lignans. Analysis of Figures 1-3 shows that improved resolution was obtained within all three groups of compounds by oplc when compared to tlc. Further resolution was possible by oplc using hptlc plates, as evidenced by the separation of the simaroubolides glaucaroubolone and 6α-senecioyloxychaparrin in this manner. Development times were 3-8 times shorter by oplc than in the tlc separations used in this investigation.

Oplc will probably be of most interest to researchers working on natural products with antineoplastic and other types of biological activity, because of its pre-

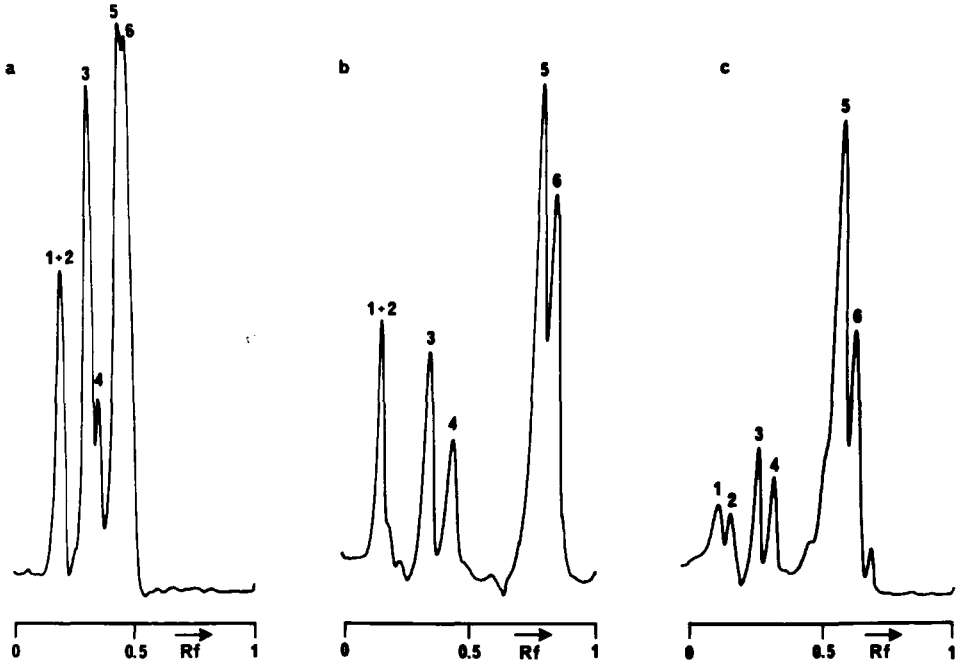

FIGURE 1. Analytical separation of simaroubolides. Peaks: 1=glaucaroubolone, 2=6α-senecioyloxychaparrin, 3=chaparrinone, 4=holacanthone, 5=6α-senecioyloxychaparrinone, 6=isobrucein A. (**a**) Normal tlc: mobile phase, $CHCl_3$-EtOH-EtOAc-hexane (70.0:9.5:18.6:1.9); development time, 100 min; (**b**) oplc with tlc plate: mobile phase, $CHCl_3$-EtOH-EtOAc-hexane (51.2:7.0:13.6:28.2); development time, 12 min (continuous development); flow rate, 0.4 ml/min; (**c**) oplc with hptlc plate: mobile phase, development time, and flow rate, as for (**b**).

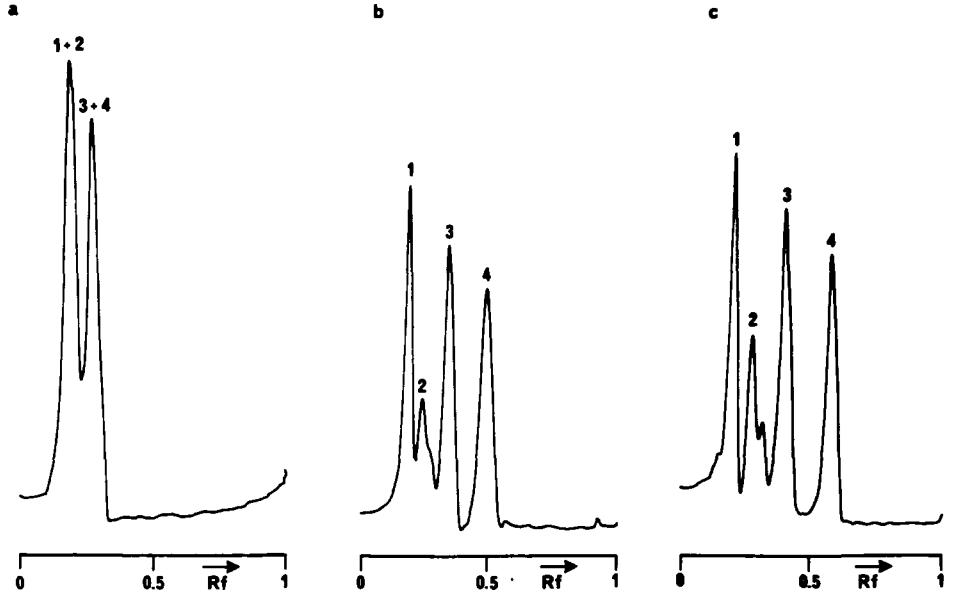

FIGURE 2. Analytical separation of podophyllotoxin-type lignans. Peaks: 1=α-peltatin, 2=podophyllotoxin, 3=β-peltatin; 4=4′-demethyldeoxypodophyllotoxin. (**a**) Normal tlc: mobile phase, $CHCl_3$-EtOH-EtOAc-hexane (18.3:5.8:34.1:41.8); development time, 65 min; (**b**) oplc with tlc plate: mobile phase, $CHCl_3$-EtOH-EtOAc-hexane (9.2:2.9:17.0:70.9); development time, 8 min (continuous development); flow rate, 0.8 ml/min; (**c**) oplc with hptlc plate: mobile phase, development time, and flow rate, as for (**b**).

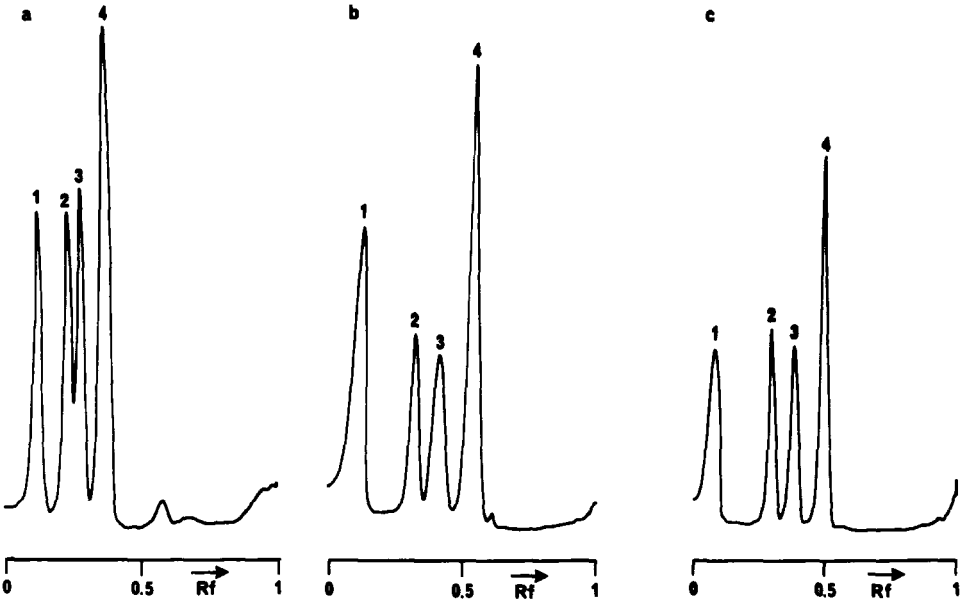

FIGURE 3. Analytical separation of *Camptotheca* alkaloids. Peaks: 1=10-hydroxycamptothecin, 2=10-methoxycamptothecin, 3=camptothecin, 4=9-methoxycamptothecin. (**a**) Normal tlc: mobile phase: THF-CH_2Cl_2 (1:3); development time, 60 min; (**b**) oplc with tlc plate: mobile phase, as in (**a**); development time, 20 min; flow rate, 0.2 ml/min; (**c**) oplc with hptlc plate: mobile phase, development time, and flow rate, as for (**b**).

parative application. Typically, the final purification of milligram quantities of biologically active components of plant-derived fractions by preparative tlc or column chromatography is rather time-consuming and can sometimes be inefficient. Using the optimized solvent system $CHCl_3$-EtOH-EtOAc-hexane (20.0:9.5:65.2:5.3), impure fractions containing holacanthone (65 mg total) and glaucaroubolone (200 mg) from *Soulamea soulameoides* (12) have been purified by oplc. Pure holacanthone (54 mg) was recovered within 60 min, while glaucaroubolone (95 mg) was obtained within 80 min, using mobile phase flow rates of 1.0 and 1.5 ml/min, respectively. The separation obtained and conditions used for the preparative purification of a mixture of α-peltatin and podophyllotoxin by oplc are shown in Figure 4. It may be noted that since this is an on-line technique, no scraping or eluting of bands was necessary, and the eluates were collected as fractions after chromatography. It was, however, found necessary to precondition plates for preparative oplc with hexane, prior to use, to remove air from the system. Also, a linear flow migration was ensured by scraping out an inlet trough on the oplc plate.

In conclusion, as shown by the analytical and preparative separations obtained in this investigation, we feel that oplc is well worthy of being included in the armamentarium of techniques available to the natural product chemist for the separation of compounds with biological activity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Oplc separations were carried out on a Chrompres 25 Oplc system (Labor MIM, H-1445, P.O. Box 280, Budapest, Hungary). Plate-edge impregnation for oplc was performed with IMPRESS II polymer suspension (Labor MIM). The cushion pressure during separation was kept at 25 bar (360 psi). All solvents used were analytical grade.

TEST COMPOUNDS.—A total of 14 antineoplastic natural products, representing three compound classes, were utilized in this investigation. The simaroubolides glaucaroubolone, holacanthone, and isobrucein A were isolated from *Soulamea soulameoides* (Gray) Nooteboom (12),

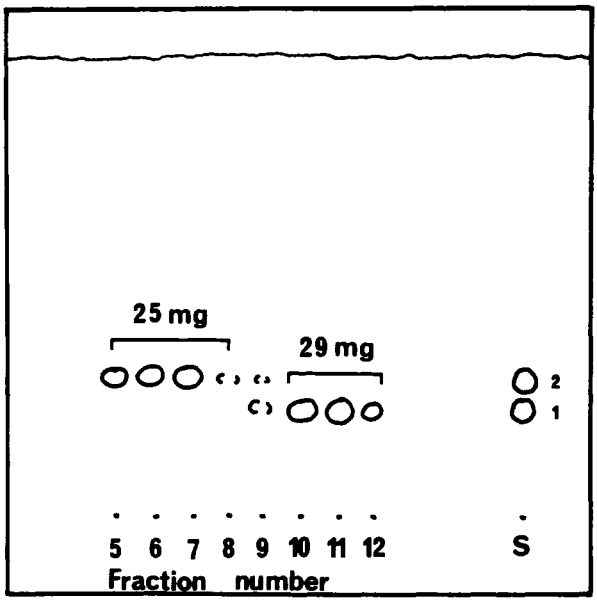

FIGURE 4. Thin-layer chromatogram showing preparative separation of 60 mg of a mixture of α-peltatin (**1**) and podophyllotoxin (**2**) by oplc. Mobile phase: Me_2CO-hexane (1:1); development time, 120 min; flow rate, 1.5 ml/min. A total of 15 fractions (5-15 ml each) were collected. Podophyllotoxin (25 mg) was obtained pure in fractions 5-8, while pure α-peltatin (29 mg) occurred in fractions 10-12.

while the other quassinoids used were chaparrinone, 6α-senecioyloxychaparrin, and 6α-senecioyloxychaparrinone, from *Simaba multiflora* A. Juss (13). Of four lignans represented, α-and β-peltatins and podophyllotoxin were extracted from *Linum album* Kotschy ex Bois. (14), and 4′-demethyldeoxypodophyllotoxin was a constituent of *Amanoa oblongifolia* Muell. Arg. (15). Two of four *Camptotheca* alkaloids employed, namely, camptothecin and 9-methoxycamptothecin, were isolated from *Merrilliodendron megacarpum* (Helms.) Sleum. (16), and 10-hydroxycamptothecin and 10-methoxycamptothecin were provided as gifts by Dr. J.D. Douros. Crude samples of glaucaroubolone and holacanthone, and a mixture of podophyllotoxin and α-peltatin, that were used for preparative studies, were also obtained from the above-mentioned plant sources.

ANALYTICAL SEPARATIONS.—Separations were conducted on precoated tlc and hptlc silica gel 60 F_{254} aluminum-backed plates (Merck, Darmstadt, W. Germany). No plate-edge impregnation was carried out on plates used for tlc. Plate dimensions were 10×20 cm (0.2 mm layer thickness) in all cases. For tlc developments, the separation pathway used was 18 cm, while a separation pathway of 17 cm was used for oplc experiments. For oplc performed with tlc and hptlc plates, plates were preconditioned by flushing with hexane for 5 min at rates of 1.55 and 0.4 ml/min, respectively, to displace air in the absorbent.

Solute mixtures used in tlc and optlc experiments were constituted with equal quantities of each component of each mixture (final concentration ca. 1 mg/ml) and were applied in 5-30 μg quantities. Evaluation of chromatograms was conducted using a Zeiss KM 3 chromatogram spectrophotometer (Zeiss, Oberkochen, W. Germany). Uv detection was carried out at 254 nm for the simaroubolide and lignan mixtures and 330 nm for the *Camptotheca* alkaloids.

PREPARATIVE SEPARATIONS.—Precoated PSC-silica gel 60 F_{254}S plates, 20×20 cm, 1 mm layer thickness, with a preconcentrating zone (Merck), were used for all preparative oplc separations. Prior to development, an additional inlet trough was scraped into the preadsorbent to flash dispense the solvent and all plates were preconditioned with hexane at 3 ml/min for 25 min.

ACKNOWLEDGMENTS

Isolation and identification studies on most of the antineoplastic agents investigated herein were funded by grant CA-33047 and contracts CM-22078 and CM-97295 from the National Cancer

Institute, NIH, Bethesda, Maryland. We are grateful to the various postdoctoral research associates and graduate students who obtained these isolates in this laboratory. We also wish to acknowledge Dr. J.D. Douros, formerly of the National Cancer Institute, for samples of 10-hydroxycamptothecin and 10-methoxycamptothecin, and Mr. J.W. Newman, of Newman-Howells Associates, Winchester, Hants., UK, for the loan of a Chrompres 25 oplc system, while this study was in progress.

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Received 30 July 1986