72. Direct Obtention of Pure Compounds from Crude Plant Extracts by Preparative Liquid Chromatography

by **Kurt Hostettmann**[1]**, Michael J. Pettei, Isao Kubo** and **Koji Nakanishi**
Department of Chemistry, Columbia University, New York, New York 10027, USA

(1. II. 77)

Summary

It has been possible to submit the crude hexane extract of the barks of a medicinal plant directly to preparative liquid chromatography. A two step solvent elution gave 6 peaks, four of which readily afforded crystalline material. The entire process required 2–3 hours whereas conventional chromatography required *ca.* 2 weeks.

1. Introduction. – In connection with our isolation and structural studies on insect antifeedants [1] and other biologically active compounds from African plants[2], a large number of species have been collected. The first step in such studies, or more generally in natural products studies, is usually to extract the plant with solvents of increasing polarity and then to submit each solvent extract to further chromatography using appropriate bio-assays for the detection of bioactive components. Frequently, the column chromatographic separation is a very tedious and time-consuming process due to complexity of the crude mixture.

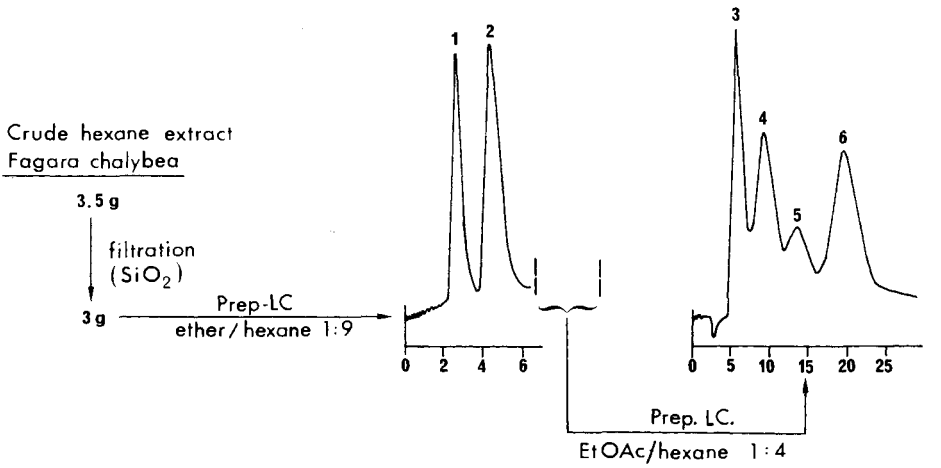
In the following, we report the direct usage of preparative scale liquid chromatography for the separation of pure constituents from a crude plant extract containing terpenoids and alkaloids. This has resulted in deletion of the laborious conventional chromatographic step and a great simplification of the entire isolation process.

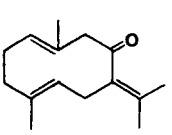
2. Results. – The dried bark (150 g) of the well-known Indian and African medicinal plant *Fagara chalybea* ENGL. (*Rutaceae*) [2] was extracted with hexane. Thin-layer chromatography showed the extract to consist of many components with a wide range of polarity, including a very polar fraction which remained at the starting point of the plate.

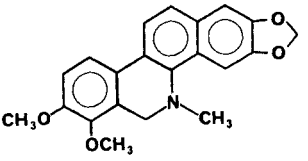
In order to avoid column contamination, this small amount of polar material was removed from the bulk of the extract by filtration through silica gel with ether/hexane 1:1. As shown in *Scheme 1*, the filtrate was submitted to preparative chromatography upon which the least polar fractions **1** (~ 1 g) and **2** (850 mg) were obtained in 6 minutes. After peak **2**, the solvent system was switched to the more polar ethyl

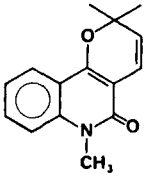
[1] On leave from Institut de Chimie, Université de Neuchâtel, CH-2000 Neuchâtel.

[2] The studies were initiated during the stay of *Isao Kubo* at the *International Center of Insect Physiology and Ecology* (ICIPE), Nairobi, Kenya.









1 mixture (1 g)
2 germacrone (850 mg)
3 dihydrochelerythrine (120 mg)
6 N-methylflindersine (125 mg)
4 mixture (180 mg)
5 pure compound (40 mg)

acetate/hexane (1:4) mixture, and the remainder was collected in one fraction. This fraction was concentrated, and a second stage prep. LC. with the above solvent system was carried out. This resulted in the separation of 4 fractions: **3** (120 mg), **4** (180 mg), **5** (40 mg), and **6** (125 mg) in 25 minutes. As monitored by TLC., fractions **2**, **3**, **5** and **6** were pure and readily afforded crystalline material after one or two recrystallizations. Fraction **1** was a viscous yellow oil probably containing long-chain hydrocarbons and other non-polar materials, whereas fraction **4** still contained several components.

Spectroscopic studies of the pure compounds led to the following structures: **2**, germacrone; **3**, dihydrochelerythrine [3]; and **6**, N-methylflindersine, a new alkaloid[3]). The structure determination of compound **5** is currently under investigation, and will be published shortly along with other data [4].

[3]) This *N*-methyl derivative has been made during the course of the structure determination of flindersine; *R.F.C. Brown, J.J. Hobbs, G.K. Hughes, & E. Ritchie*, Austral. J. Chemistry 7, 348 (1954).

3. Discussion. – A separation of the same scale by conventional column chromatography not only took two weeks but also required usage of repeated chromatographies under different conditions including gradient elution. The prep. LC. method thus results in a great reduction of time, and as a consequence the risk of sample deterioration on the column is also minimized. For example, the relative amount of dihydrochelerythrine **3** obtained by conventional chromatography is much less. This is presumably due to the fact that owing to its instability it is readily oxidized to the quaternary ammonium salt chelerythrine [5]. Furthermore, no compound corresponding to peak **5** was obtained by usual chromatographic separation; again compound **5** is unstable and is readily oxidized to a more polar compound. Application of this method to the more polar extracts are in progress; these results will be reported later since they require the preparation of bonded-type column packing material. The present method can also be applied to leaf extracts, but in this case it would be useful to remove the chlorophylls, waxes, *etc.*, by treatment with Sephadex LH-20 prior to injection [6].

The present work was supported by *NIH* grant AI 10187. One of the authors (*K.H.*) expresses his gratitude to the *Fonds National Suisse de la Recherche Scientifique* for financial support (grant no. 820.425.76).

Experimental Part

The plant material was collected near Mombasa, Kenya. The dried bark (150 g) was extracted with 1 l cold hexane (3 ×). After evaporation of the solvent, the remaining sticky oil (~3.5 g) was filtered through 100 g of silica gel using ether/hexane 1:1.

Separations were performed on a *Waters* Prep. LC-500 instrument equipped with a refractive index detector. This apparatus utilizes a pressure chamber (400 psi or 27 atm. of N_2) to compress silica gel cartridges (30 cm × 5 cm), thus creating a column of relatively high efficiency. For the flow rate employed, *i.e.*, 250 ml/min, a solvent pressure of less than 100 psi (7 atm.) was needed. Conditions for the individual runs are as follows:

Run 1. Sample: 3 g of extract in 10 ml of hexane solution; Solvent: ether/hexane 1:9; Detector Sensitivity: 5 ×.

Run 2. Sample: 0.6 g in 5 ml hexane; Solvent: EtOAc/hexane 1:4; Detector Sensitivity: 50 ×.

REFERENCES

[1] *P. R. Zanno, I. Miura, K. Nakanishi & D. L. Elder*, J. Am. chem. Soc. *97*, 1975 (1975); *I. Kubo, I. Miura & K. Nakanishi*, J. Am. chem. Soc. *98*, 6704 (1976); *I. Kubo, Y.-W. Lee, V. Balogh-Nair, K. Nakanishi & A. Chapya*, J. chem. Soc. Chem. Commun. *1976*, 949; *I. Kubo, S. P. Tanis, Y.-W. Lee, I. Miura, K. Nakanishi & A. Chapya*, Heterocycles *5*, 485 (1976); *I. Kubo, Y.-W. Lee, M. Pettei, F. Pilkiewicz & K. Nakanishi*, J. chem. Soc. Chem. Commun., in press (1976).

[2] *J. O. Kokwaro*, 'Medicinal Plants of East Africa,' East African Literature Bureau, Nairobi 1976; *J. M. Watt & M. G. Breyer-Brandwijle*, 'The Medicinal and Poisonous Plants of Southern and Eastern Africa,' E. & S. Livingstone, London 1962.

[3] *P. J. Scheuer, M. Chang & C. Swanholm*, J. org. Chemistry *27*, 1472 (1962).

[4] *F. Yeh, I. Miura, K. Hostettmann, I. Kubo & K. Nakanishi*, to be published.

[5] *H. R. Arthur, W. H. Hui & Y. L. Ng*, J. chem. Soc. *1959*, 1840.

[6] *K. Hostettmann & A. Jacot-Guillarmod*, J. Chromatogr. *124*, 381 (1976).