Journal of Chromatography, 265 (1983) 97–104
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,821

APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR ANALYSIS AND ISOLATION OF SESQUITERPENE LACTONES

BERNARD MARCHAND, H. MOHAN BEHL and ELOY RODRIGUEZ*
Phytochemical Laboratory, Ecology and Evolutionary Biology, University of California, Irvine, CA 92717 (U.S.A.)
(Received March 1st, 1983)

SUMMARY

The capacity of a reversed-phase high-performance liquid chromatographic system using Ultrasphere-ODS columns and a gradient of acetonitrile–water to separate sesquiterpene lactones has been studied. Retention times and capacity factors of 33 compounds of the pseudoguaianolide and xanthanolide skeletal types occurring in *Parthenium* (Asteraceae) are reported. Crude extracts of *Parthenium schottii* containing a mixture of sesquiterpene lactones have been analyzed and separated using the high-performance liquid chromatographic system.

INTRODUCTION

Sesquiterpene lactones are characteristic constituents of the Asteraceae but also occur sporadically in other angiosperm families like Lauraceae, Magnoliaceae and Umbelliferae[1]. During the past three decades about 1000 sesquiterpene lactones have been isolated, identified and, in some cases, synthesized[2]. The information about their bioactive properties is gradually building up. Over 50 compounds have been evaluated for their growth inhibitory potential against numerous tumor models[3]. Kupchan *et al.*[4], Hanson *et al.*[5] and Lee *et al.*[6] have reported many sesquiterpene lactones from species of *Ambrosia, Artemesia, Eupatorium, Elephantopus, Helanium, Encelia* and *Vernonia* that exhibit antitumor and cytotoxic activity. Selective alkylation of enzymes controlling cell division was suggested as the mechanism of action. Sesquiterpene lactones have also been shown to have schistosomicidal properties[7]. Helenalin from species of *Helanium* was shown to exhibit activities against human pathogenic fungi[8]. Growth of *Staphylococcus aureus* and *Candida albicans* (yeast) was inhibited by lactones from *Mikemia monagasensis*[9]. Parthenin from *Parthenium hysterophorus* was reported to be toxic to sporangial germination in *Sclerospora graminicola*[10]. Many sesquiterpene lactones, for instance in *Parthenium, Ambrosia, Chrysanthemum* and *Frullania*, are known to cause allergic contact dermatitis and constitute a major class of allergens[11]. Resistance to insect feeding[12,13] and plant growth regulation[14] are other biological activities of these compounds. The toxic effect of hymenovin on sheep and goats is one example of their well known vertebrate poisoning properties[15].

0021-9673/83/$03.00

In this study, we selected *Parthenium* (Asteraceae) for isolation and analysis of sesquiterpene using high-performance liquid chromatography (HPLC). As in many other plant taxa, sesquiterpene lactones have been used to establish chemotaxonomical and ecogeographical relationships in at least twenty species of *Parthenium*[16] and *Ambrosia*[17]. As a result of the renewed interest in natural rubber and other chemical specialties, guayule *(P. argentatum)* and its relatives are receiving considerable attention. In order to improve the guayule crop for rubber and resin yields, research has been undertaken in the U.S.A. to hybridize it with more robust species like *Parthenium tomentosum, P. fruticosum* and *P. schottii* for higher biomass; and *P. rollinsianum, P. alpinum* and *P. integrifolium* for cold tolerance. Resin of all species of *Parthenium* other than guayule and *P. rollinsianum* constain sesquiterpene lactones. In *Parthenium,* only pseudoguaianolide and xanthanolide type skeletons have been reported[18]. Sesquiterpene lactones reported in various species of *Parthenium* are presented in Fig. 1.

Earlier studies on isolation and identification of these compounds have been mainly qualitative and restricted to thin-layer and customary chromatography[19–22]. HPLC appears to be a method of choice for quick, comparative and quantitative analysis. It provides an added advantage of working with very small amounts of material. Gas chromatography could be equally useful; however, except in a few cases[23–25], it remains of limited use because the compounds are not always volatile enough and need derivatization[26–28]. There have been very few attempts to use HPLC for studying this group of natural products in analytical[29] and preparative scale[30,31]. The ability of a reversed-phase HPLC system to separate all reported sesquiterpene lactones of the genus *Parthenium* has been investigated in this study. The efficiency of this system is demonstrated by analysing a crude extract of *Parthenium schottii.*

EXPERIMENTAL

Apparatus

Analysis was performed on Beckman gradient liquid chromatograph series 334 equipped with a 421 controller, 110 A pumps and 210 sample injection valve, and fitted with 20- or 250-μl loops. Detection was achieved using a Hitachi 110-10 variable-wavelength detector. The chromatograms were recorded on a Corning recorder 840. Retention times were obtained with a Shimatzu Model C-E1B integrator. Chromatographic columns consisted of either an Ultrasphere-ODS column (150 × 4.6 mm) or an Ultrasphere-ODS preparative column (250 × 10 mm) equipped with an Altex precolumn (45 × 4.6 mm).

Elution

Two solvents were used: acetonitrile (A) and water (B). The elution profile for the analytical column was as follows: 0–20 min, 10–25% A (linear gradient); 24–27 min, 25–40% A (linear gradient); and 40% A maintained after 27 min. In the preparative column, the system was modified as follows: 0–18 min, 25% A (isocratic); 18–21 min, 25–40% A (linear gradient); and 40% A from 21 to 36 min (isocratic). The flow-rate was 1 ml/min for the analytical column and 4 ml/min for the preparative column.

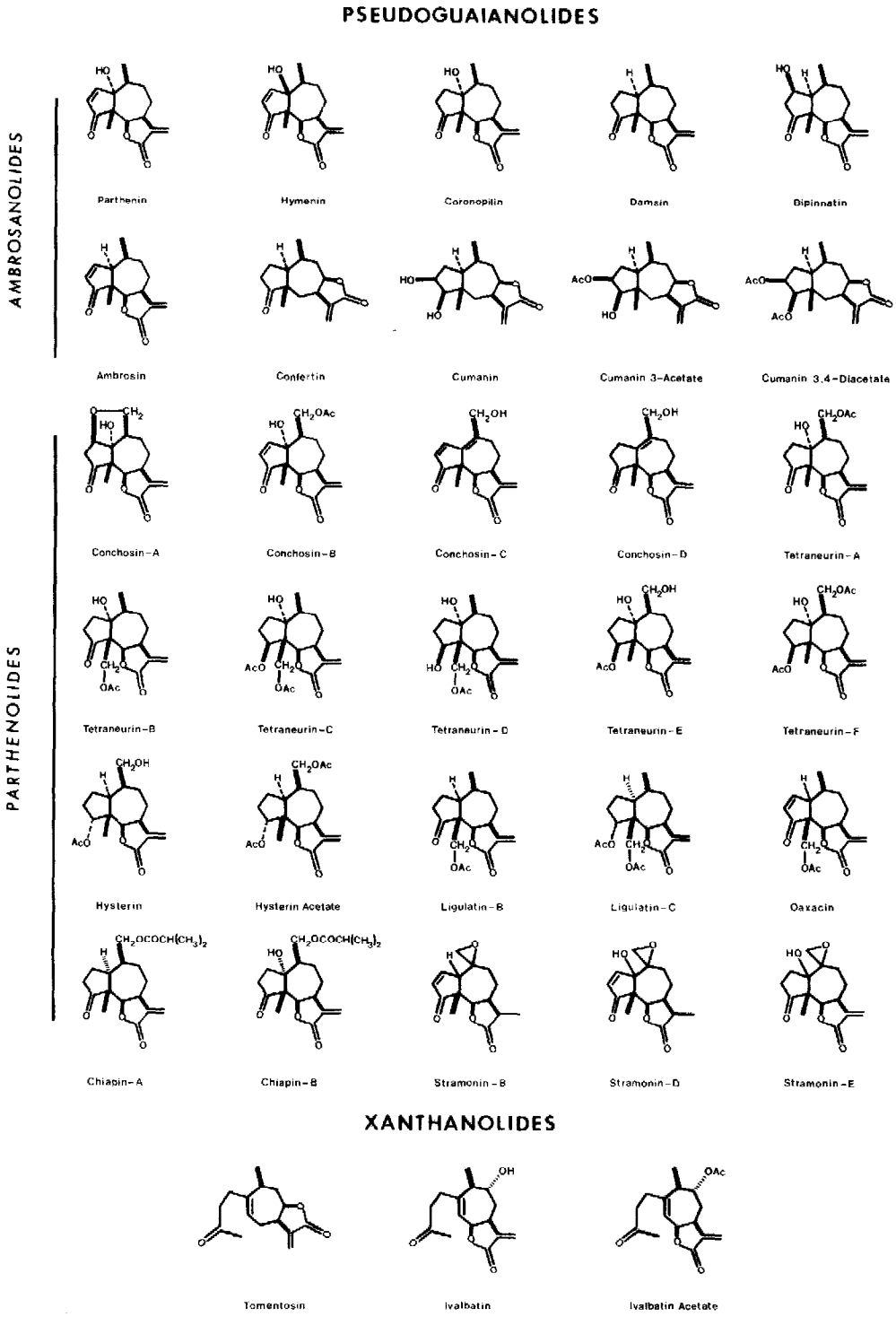

Fig. 1. Sesquiterpene lactones from *Parthenium*.

TABLE I

RETENTION TIME, CAPACITY FACTOR AND PLANT SOURCES OF SESQUITERPENE LACTONES

t_R = Retention time; k' = capacity factor.

Sesquiterpene No.	*Substance*	*t_R (min)*	*k'*	*Source reference**	*Plant source*
1	Tetraneurin E	8.37	3.25	1	*Parthenium confertum, P. integrifolium, P. fruticosum* var. *trilobatum*
2	Conchosin A	8.44	3.28	1	*P. confertum* var. *microcephalum*
3	Tetraneurin C	11.35	4.76	1	*P. alpinum, P. fruticosum* var. *trilobatum, P. integrifolium, P. lozanianum*
4	Ivalbatin	11.82	5.00	2, 3	*Iva dealbata, P. incanum* (Nevada)
5	Hymenin	13.61	5.91	1	*Hymenoclea salsola, P. confertum*
6	Tetraneurin A	13.88	6.04	1	*P. alpinum, P. cineraceum, P. confertum, P. fruticosum*
7	Tetraneurin D	14.56	6.39	1	*P. lozanianum, P. fruticosum P. schottii*
8	Conchosin B	14.75	6.48	1	*P. confertum*
9	Cumanin	15.65	6.94	2	*P. incanum* (Nevada), *Ambrosia artemisiifolia, A. psilostachya*
10	Tetraneurin B	16.04	7.14	1	*P. alpinum, P. fruticosum, P. ligulatum, P. lozanianum, P. schottii*
11	Hysterin	16.49	7.37	1	*P. bipinnatifidum, P. confertum*
12	Parthenin	18.78	8.53	1	*P. hysterophorus, Iva nevadensis*
13	Ligulatin C	18.99	8.64	1	*P. tomentosum* var. *tomentosum, P. cineraceum*
14	Coronopilin	19.73	9.01	1	*A. psilostachya, A. dumosa, A. arborescens, A. artemisiifolia, P. schottii, P. incanum, Hymenoclea salsola, Iva acerosa, I. nevadensis, Cyclochaena xanthifolia*
15	Bipinnatin	20.55	9.43	1	*P. bipinnatifidum*
16	Oaxin	26.71	12.55	1	*P. tomentosum* var. *tomentosum*
17	Ambrosin	27.11	12.76	1	*A. maritima, A. cumanensis, A. hispida, A. jamaicensis, A. psilostachya, Iva xanthifolia, P. bipinnatifidum, P. incanum, Hymenoclea salsola, H. monogyra*
18	Stramonin D	27.18	12.79	1	*P. tomentosum* var. *stramonium*
19	Tetraneurin F	27.23	12.82	1	*P. confertum*
20	Cumanin 3-acetate	27.57	12.99	2	*A. psilostachya, P. incanum* (Nevada)
21	Stramonin E	28.49	13.46	1	*P. tomentosum* var. *stramonium*
22	Ligulatum B	30.08	14.26	3	*P. tomentosum* var. *tomentosum, P. incanum, P. ligulatum*
23	Chiapin A	30.31	14.38	1	*P. fruticosum* var. *fruticosum*
24	Chiapin B	30.39	14.42	1	*P. fruticosum* var. *fruticosum*
25	Conchosin C	30.45	14.45	1	*P. confertum*
26	Conchosin D	30.50	14.48	1	*P. confertum*
27	Ivalbatin acetate	30.78	14.62	2, 3	*P. fruticosum* var. *trilobatum, P. incanum* (Nevada)
28	Stramonin B	31.83	15.15	1	*P. tomentosum* var. *stramonium*
29	Cumanin 3,4-diacetate	33.57	16.04	2	*P. incanum* (Nevada), *A. psilostachya*
30	Hysterin acetate	33.73	16.12	1	*P. bipinnatifidum*
31	Confertin	33.85	16.18	1	*P. schottii, A. confertiflora*
32	Damsin	34.14	16.33	1	*A. maritima, A. hispida, A. cumanensis, A. ambrosiodes, A. arborescens, A. chenopodiifolia, A. deltoidea, A. jamaicensis, P. bipinnatifidum,*
33	Tomentosin	35.52	17.03	1	*P. tomentosum* var. *tomentosum, Inula helenium, I. royleana*

* Source references: 1 = E. Rodriguez, sample collection; 2 = *Parthenium incanum* (Neveda), H. M. Behl, B. Marchand and E. Rodriguez, submitted for publication; 3 = *Parthenium tomentosum* var. *tomentosum* compounds provided by Dr. Ortega (Mexico).

Detection

The UV detector was set at 215 nm. This wavelength was found to be a reasonable average of λ_{max} for all sesquiterpene lactones investigated.

Samples

Standards were dissolved in methanol and applied to the column. The sources of all sesquiterpene lactones are given in Table I. Leaves and inflorescences from two-year-old *P. schottii* plants growing in a greenhouse were collected. The specimens have been deposited in the University of California Riverside herbarium. Samples (100 g) of the air-dried materials were extracted in 500 ml of chloroform using a tissue homogenizer (Brinkman Instruments) for 5 min. The filtrate was evaporated and dissolved in 100 ml of methanol. The suspension was filtered through Millipore before injection.

RESULTS AND DISCUSSION

The retention times of 33 sesquiterpene lactones occurring in *Parthenium*, are reported in Table I. As the retention times of most of the compounds are between 14 and 31 min, a mixture of sixteen sesquiterpene lactones covering all range of polarity was selected to present a single run separation (Fig. 2). As no particular species of *Parthenium* is reported to have more than seven sesquiterpene lactones, there is a little likelihood that some compounds would overlap in a particular taxon using the suggested system.

In the initial approach to this system, RP-18 and RP-8 analytical columns were used. In order to use HPLC at preparative scale, we developed a system using an

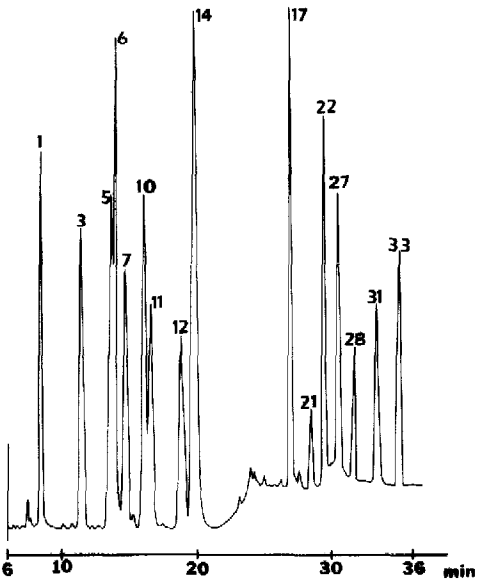

Fig. 2. HPLC separation of sixteen sesquiterpene lactones from *Parthenium*. Numbers correspond with those in Table I.

RP-18 column of larger diameter (10 mm). As reported in the experimental part, it was necessary to increase the flow-rate and slightly modify the elution system for the preparative column. As observed, an equally good separation was achieved by using relatively higher initial concentrations of acetonitrile. This system even proved to give a better resolution for "stubborn pairs" such as tetraneurin A and tetraneurin D, previously not separated in the analytical column. In order to complete analysis within a reasonable time period, the non-polar compounds were eluted in both systems by rapidly increasing the amount of acetonitrile (40% in 3 min). However, the separation could be further improved by decreasing the slope of the gradient.

The retention time of each compound could be explained as a result of the competition between hydrophobic interactions with the stationary phase and hydrogen bonding with the solvent. The basic skeleton of the sesquiterpene lactones investigated being a common feature, different degrees of oxidation in C_1, C_2, C_4, C_{14} and C_{15} positions are pertinent to explain the sequence of elution. As expected, damsin eluted very late (34.14 min) from the column. When an OH group is present in position C_1, the oxygen being a hydrogen bond acceptor, the retention time of coronopilin happens to be 14.41 min less than damsin. If the carbonyl in position C_4 becomes α,β unsaturated, this leads to a slight increase of polarity which explains the difference of 0.95 min between the coronopilin and parthenin retention times. The oxidation of C_{15} increases the polarity much more and effects the retention time by a few minutes (difference between parthenin and conchosin B is 4.03 min). The same change of polarity could be expected when C_{14} is oxidized and thus a difference of 4.06 min in retention times between damsin and ligulatin B was observed. The higher polarity of tetraneurin E *versus* tetraneurin F is probably due to the alcohol which behaves as a better hydrogen bond attractor than the carbonyl group. However, in

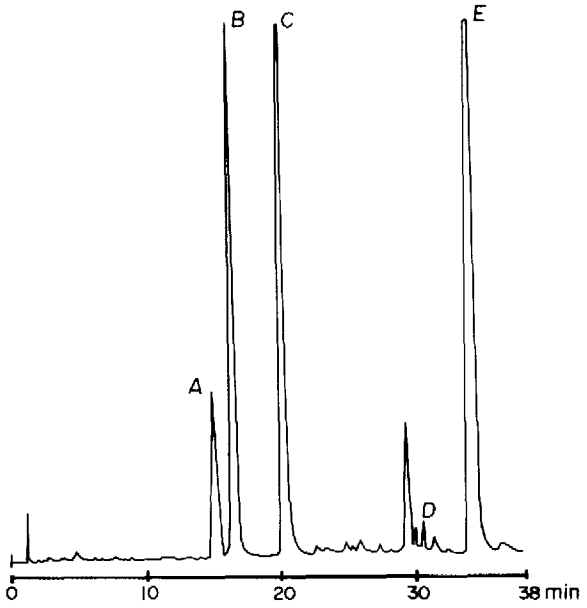

Fig. 3. HPLC chromatogram of crude extract of *P. schottii*. Peaks: A = tetraneurin D; B = tetraneurin B; C = coronopilin; D = ligulatin B; E = confertin.

TABLE II

SESQUITERPENE LACTONE ANALYSIS OF *P. SCHOTTII*

Compound	*Retention time (min)*	*Concentration (mg/g dry weight)*
Tetraneurin D	14.66	3.9
Tetraneurin B	15.99	8.9
Coronopilin	19.77	29.2
Ligulatin B	30.00	0.5
Confertin	33.83	57.4

some cases a strong internal hydrogen bond can be formed between an OH and a carbonyl group resulting in lack of interaction with the solvent. An example of this phenomenon is the smaller retention time of tetraneurin C. Following the above general "rules" we were able to predict most of the relative retention times reported in this study. However, differences of polarities with only a change of configuration in C_1, as in hymenin and parthenin, could not be explained.

Sesquiterpene lactones analysis of the crude extract of *Parthenium schottii* was undertaken in order to prove the efficiency of this method. The only purification required was the precipitation of waxes in methanol to avoid plugging of the column. A very neat separation of all the sesquiterpene lactones present in this species was achieved as shown in the chromatogram in Fig. 3. Different constituents extracted using this preparative technique were identified as tetraneurin B, tetraneurin D, coronopilin, ligulatin B and confertin. The structures were confirmed by the ^1H nuclear magnetic resonance and infrared spectra and were found to be identical to those of known standards. The sesquiterpene lactone profile from this species was in accordance with an earlier report by Rodriguez *et al.*[33]. Other components detected in HPLC chromatogram were isolated but proved not to contain any lactone moiety. The reported sesquiterpene lactones were quantified by HPLC using external standards; the results are reported in Table II.

The HPLC system suggested in this study can be used to detect and identify new compounds present even in small amounts in various species of *Parthenium*. As it is far less time consuming and more precise than routine chromatography, it provides a good tool for quantification of sesquiterpene lactones in different parts of plants or seasonal variation studies. As the compounds investigated in the system cover a wide range of polarity, sesquiterpene lactones from other genera most probably could be separated following the above procedure. Experiments to screen species of *Parthenium* and *Ambrosia* are in progress in this laboratory.

ACKNOWLEDGEMENTS

We are grateful to the Council for International Exchange of Scholars (H.M.B.) and Nato (B.M.) for financial support. The project was supported by NIH Grant AI 18398-01 and NSF Guayule Grant PEM 8104620. We thank Dr. A. Ortega for supplying some of the samples and Dr. H. T. Huang (N.S.F.) for support and encouragement.

REFERENCES

1 E. Rodriguez, H. Yoshioka and T. J. Mabry, *Phytochem.*, 10 (1971) 1145.
2 N. H. Fisher, E. J. Olivier and H. D. Fisher, *Fortschr. Chem. Org. Naturst.*, 38 (1978) 48.
3 J. L. Hartwell and B. J. Abbott, *Advan. Pharmacol. Chemother.*, 7 (1969) 117.
4 S. M. Kupchan, M. A. Eakin and A. M. Thomas, *J. Med. Chem.*, 14 (1971) 1147.
5 R. L. Hanson, M. A. Lardy and S. M. Kupchan, *Science*, 168 (1970) 368.
6 K. H. Lee, E. S. Juang, C. Piantadosi, J. S. Pagano and T. A. Geissman, *Cancer Res.*, 31 (1971) 1649.
7 W. Vichnewski, S. J. Sarti, B. Gilbert and W. Herz, *Phytochem.*, 15 (1976) 191.
8 W. Olechnowicz and S. Stepien, *Dissert. Pharm.*, 15 (1963) 17.
9 S. B. Mathur, P. G. Tello, C. M. Fermin and V. Mora-Arallano, *Rev. Latinoam. Quim.*, 6 (1975) 201.
10 M. B. S. Char and S. Shankarabhat, *Experientia*, 31 (1975) 1164.
11 J. C. Mitchell, G. Dupuis and G. H. N. Towers, *Brit. J. Derm.*, 87 (1972) 235.
12 W. C. Burnett, S. B. Jones, T. J. Mabry and W. Padolina, *Biochem. System. Ecol.*, 9 (1974) 25.
13 M. Isman and E. Rodriguez, *Annual meeting of Entomological Soc. of America and Canada, Toronto, 1982.*
14 D. Gross, *Phytochem.*, 14 (1975) 2105.
15 G. W. Ivie, D. A. Witzel, W. Herz, R. Kannan, J. O. Norman, D. D. Rushing, J. H. Johnson, L. D. Rowe and J. A. Veech, *J. Agr. Food Chem.*, 23 (1975) 841.
16 E. Rodriguez, *Biochem. System. Ecol.*, 5 (1977) 207.
17 F. C. Seaman, *Bot. Rev.*, 48 (1982) 121.
18 E. Rodriguez, *Ph.D. dissertation*, Texas University, Austin, TX, 1975.
19 A. K. Picman, R. L. Ranieri, G. H. N. Towers and J. Lam, *J. Chromatogr.*, 189 (1980) 187.
20 A. K. Picman, I. Panfil and G. H. N. Towers, *J. Chromatogr.*, 212 (1981) 379.
21 B. Drozdz and E. Blaszyk, *Planta Med.*, 33 (1978) 379.
22 B. R. Chhabra, Y. K. Mehta, R. P. Gulati and R. S. Dhillon, *Riechst. Aromen Kosmet.*, 27 (1979) 74.
23 A. Cheminat, C. Benezra, M. J. Farrall and J. M. Frechet, *Can. J. Chem.*, 59 (1981) 1405.
24 J. Froberg and G. Magnusson, *J. Amer. Chem. Soc.*, 100 (1978) 6728.
25 G. R. Jamieson, E. H. Reid, B. P. Turner and A. T. Jamieson, *Phytochem.* 15 (1976) 1713.
26 H. Pyysalo, E.-L. Seppa and K.-G. Widén, *J. Chromatogr.*, 190 (1980) 466.
27 D. L. Perry, D. M. Desiderio and N. H. Fisher, *Org. Mass Spectrom.*, 13 (1978) 325.
28 S. F. Watkins, J. D. Korp, I. Bernal, D. L. Perry, N. S. Bhacca and N. H. Fisher, *J. Chem. Soc., Perkin Trans. II*, (1978) 599.
29 D. Strack, P. Proksch and P. G. Gielz, *Z. Naturforsch. C.*, 35 (1980) 915.
30 W. M. Daniewski, M. Kocor and J. Krol, *Rooz. Chim.*, 50 (1976) 2095.
31 M. Belkouski and T. J. Mabry, *Rev. Latinoam. Quim.*, 7 (1976) 111.
32 E. Rodriguez, H. Yoshioka and T. J. Mabry, *Rev. Latinoam. Quim.*, 3 (1972) 184.