Chemistry of Natural Compounds, Vol. 29, No. 4, 1993

THE INVESTIGATION OF NATURAL COMPOUNDS BY HPLC. III. A STUDY OF SESQUITERPENE LACTONES OF THE GUAIANE TYPE BY MICROCOLUMN HPLC

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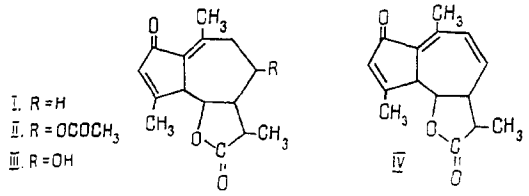
UDC 547.856:543(062):547.314

Two comparable methods of microcolumn high-performance liquid chromatography (HPLC) have been developed for the sesquiterpene lactones leucomicin, matricarin, austricin, and anhydroaustricin and for the drug isolated from the epigeal part of Artemisia leucodes.

In the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan (Tashkent), as the result of chemical investigation and pharmacological trials, an antiatherosclerotic drug has been created on the basis of sesquiterpene lactones isolated from *Artemisia leucodes*. In the present paper we give the results of a study of the composition of the drug by the HPLC method.

A detailed study has been made of the chromatographic separation of natural sequiterpene lactones of the guaiane type: leucomisin (I), matricarin (II), austricin (III), and anhydroaustricin (IV). These lactones were isolated from plants of the Compositae family, while anhydroaustricin can also be obtained by the dehydration of austricin [1-5]. It must be mentioned that facts are known concerning the genetic determinacy of the amounts of lactones in plants. Disregarding some annual fluctuations, their presence is constant in composition and amount for genetically determined species and does not depend on morphological variability [6]. Consequently, the microcolumn methods of HPLC that are being developed may have great importance for the study of the dynamics of the accumulation and biosynthesis of guaianolides in plants and for taxonomic investigations.

As is known, sesquiterpene lactones are extremely labile substances and have a reactive center — the γ-lactone ring — which is cleaved under the action of alkali with the formation of salts of a γ-hydroxy acid, while under the action of an acid the ring closes again to form the initial lactone. Furthermore, lactones may possess other reactive centers. Examples are double bonds, which are oxidized to epoxy groups, and these, in their turn, are opened in an acid medium with the formation of hydroxy groups; esterification reactions of lactones are also known [7].



The most suitable method for studying such labile compounds is reversed-phase HPLC, and we have used microcolumns filled with reversed-phase sorbents. The drug and fractions A, B, and C of sesquiterpene lactones containing mainly (II), (III), and (IV), respectively, were studied.

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Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 526-529, July-August, 1993. Original article submitted November 2, 1992.

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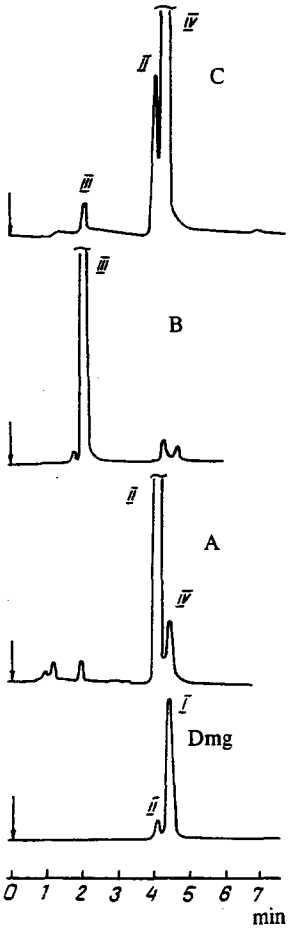

Fig. 1

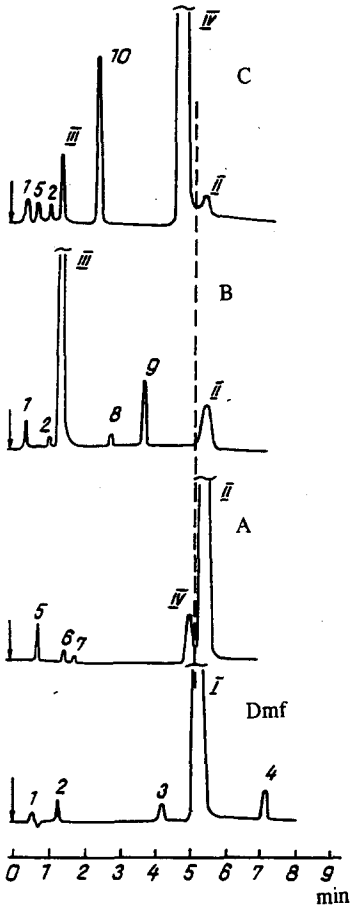

Fig. 2

Figs. 1 and 2. HPLC chromatograms of the drug and of fractions A, B, and C under the conditions of the first method (Fig. 1) and under the conditions of the second method (Fig. 2): I) leucomisin; II) matricarin; III) austricin; IV) anhydroaustricin; 1-10) unidentified minor components.

The first method of HPLC, in which an eluting system of methanol and acetate buffer was used [8], gave a good result in the separation of the lactones under study, and only leucomycin and anhydroaustricin had the same retention time in this case. Matricarin was well separated from leucomicin, and the presence of matricarin in the drug can be determined quantitatively by this method (Fig. 1).

The experimental results of HPLC showed that fraction A consisted mainly of matricarin while the second peak probably contained leucomisin or anhydroaustricin together with matricarin. Fraction A also contained a very small amount of three minor component. Fraction B consisted mainly of austricin, which was accompanied by three unknown minor substances. Fraction C contains (IV), (II), and (III), the (II) being present in considerable amounts.

In the first method of HPLC (Fig. 1), the retention times of anhydroaustricin and leucomisin coincided, and therefore the presence of anhydroaustricin in the drug and in matricarin remained unproven. To resolve this question we developed a second method of microcolumn HPLC (Fig. 2). In this variant there was a sequence of the elution of leucomisin, matricarin, and anhydroaustricin, and the peaks of leucomisin and of anhydroaustricin were displaced considerably relative to one another.

The results of the second method of HPLC showed that the drug contained no anhydroaustricin, while fraction A, consisting mainly of matricarin, also contained anhydroaustricin. Fraction B contained mainly austricin and matricarin and also four unknown minor compounds. The minor substances 8 and 9 were not present in the other fractions and the drug. Fraction C, the dominating substance of which was anhydroaustricin, had the spectra of accompanying minor compounds, some of which

TABLE 1. Amounts of Sesquiterpene Lactones and Minor Components in the Drug and in Fractions A, B, and C from the Epigeal Part of *Artemisia leucodes* Determined by the HPLC Method

Lactones and the numbers of the minor components	Amounts of the lactones and minor components, %			
	Dmg	A	B	C
I	85,64	—	—	—
II	6,62	91,12	10,77	0,68
III	—	—	79,46	4,02
IV	—	6,24	—	84,30
1	0,59	—	1,80	1,46
2	1,89	—	0,45	0,65
3	2,02	—	—	—
4	3,23	—	—	—
5	—	1,90	—	1,09
6	—	0,66	—	—
7	—	0,08	—	—
8	—	—	0,34	—
9	—	—	7,18	—
10	—	—	—	7,80

TABLE 2. Amounts of Lactones in Batches of the Substance of the Drug from *Artemisia leucodes* Determined by the HPLC Method

Lactone	Batch of the drug substance		
	240191	50691	200591
I	96,05	99,65	99,30
II	3,95	0,35	0,70

were determined in the austricin, matricarin, and leucomisin fractions. But, fraction C contained a considerable amount of an unknown lactone (peak 10) which was not found in the fractions of the other lactones and the drug.

According to the second method of HPLC, fraction C contained a smaller amount of matricarin than according to the first method. This difference shows the susceptibility of anhydroaustricin to acetylation in the presence of methanol and acetic acid on the surface of the reversed phase sorbent. As a result, part of the anhydroaustricin is converted into matricarin, and the amount of the latter rises considerably under the conditions of the first method of HPLC. In the second method of HPLC this influence on structure is excluded. Thus, the first method has basic importance for revealing the presence of matricarin in the drug, which is impossible on the use of the second method.

The second method of HPLC permitted an unambiguous answer to the question of the presence in fraction A, together with matricarin, of anhydroaustricin and not of leucomisin. This was also confirmed by the fact that as a consequence of the partial conversion of anhydroaustricin into matricarin under the conditions of the first method the quantitative ratio of matricarin and anhydroaustricin changed (from 8.78 to 11.04). However, leucomisin is not susceptible of such structural changes in either method of HPLC, which shows the presence of anhydroaustricin in fraction A.

With the aid of the two variants of HPLC it has been shown that the main component of the drug from *Artemisia leucodes* is leucomisin. The first method of HPLC revealed that the drug contained matricarin and that anhydroaustricin underwent acetylation in the presence of methanol and acetic acid on the surface of the reversed-phase sorbent as a result of which part of the anhydroaustricin was converted into matricarin. Matricarin is present in the drug in different amounts depending on the batch of the substance (Table 2).

It was shown by the second method of HPLC that the drug contained no anhydroaustricin and austricin, while ten substances of minor nature from *Artemesia leucodes* were revealed.

EXPERIMENTAL

The sesquiterpene lactones austricin, anhydroaustricin, leucomisin, and matricarin were isolated from the plant and purified by the classical methods of column liquid chromatography [2-5]. Microcolumn HPLC was conducted on a Milikhrom instrument of the Nauchpribor Production Combine (Orel). For the chromatographic separation we used a microcolumn (2 × 62 mm) of KAKh-2.

In the first variant, chromatography was conducted with the mobile phase MeOH–0,5 M acetate buffer (70:30). The second method was conducted with the mobile phase THF–0.01 M phosphate buffer (55:45). The rate of elution in the first case was 100 μl/min, and in the second case 50 μl/min. Spectrophotometry was carried out at 254 nm. The chromatograms were read by an automatic recorder. The speed of the chart strip was 3 mm/min.

The sample was introduced by means of a Milikhrom injector in a volume of 0.5-1 μl. Quantitative estimates were calculated from the areas of the peaks by the usual method. Known substances were identified by the methods of additives and by a comparison of their UV spectra with those of the individual substances.

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