Journal of Chromatography, 195 (1980) 425–430
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,823

Note

High-performance liquid chromatographic separation and analysis of steroidal constituents of two solanaceous plants

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(Received February 26th, 1980)

Various plants of the Solanaceae family are known to contain oxygenated steroids built on a C_{28} ergostane-type skeleton, such as withanolides[1], physalins[2], Nic derivatives[3–5] and the ixocarpalactones[6].

Withanolide E (Fig. 1, 1)[1] and its 4β-hydroxy derivative (2)[7,8] were found to be active against several tumour systems used in cancer chemotherapy screening, namely P338[1] and L1210[8] leukaemia, Lewis lung carcinoma and B-16 mouse melanoma[1].

In this paper, we report the application of an efficient, high-sensitivity high-performance liquid chromatographic (HPLC) method for the qualitative and quantitative analysis of two solanaceous plants: (a) *Physalis peruviana* L.[7,9] and (b) *Nicandra physaloides* (L.) Gaerth var. *albiflora*[10]. The former was found to contain steroidal lactones of the withanolide group with a 17α-oriented side-chain: withanolide E (1), 4β-hydroxywithanolide E (2), 2,3-dihydrowithanolide E (3)[7] and withanolide S (4)[9]. *Nicandra physaloides* (L.) Gaerth var. *albiflora* was found to contain two closely related steroids, namely nicalbin A (5) and nicalbin B (6). One of the interesting features of nicalbin A (5) is that the hemiacetalic hydroxyl group is axially oriented. Upon extraction with methanol or ethanol, the two epimeric C26-methyl acetals (7 and 8) and ethyl acetals (9 and 10), respectively, were obtained.

It should be emphasized that so far this is the only variety of this plant which does not contain Nic-1 (nicandrenone) (11), characterized by an expanded and aromatic ring D. This compound was a common constituent in all plants of *Nicandra physaloides* investigated by Begley *et al.*[3], Bates and Eckert[4] and our group[5].

EXPERIMENTAL

The HPLC apparatus was a Waters Assoc. Model 204 liquid chromatograph, equipped with a U6K injector, 6000A pump and Model 450 variable-wavelength detector. The column (stainless steel, 250 × 4.6 mm I.D.) was packed with LiChrosorb SI-100, 5 μm. The eluents were 2-propanol (Frutarom, Haifa, Israel), acetonitrile (spectrograde, BDH, Poole, Great Britain) and methylene chloride (BDH). The methylene chloride distilled and then passed through a 60 × 2 cm I.D. column, the bottom half of which was filled with silica gel 60

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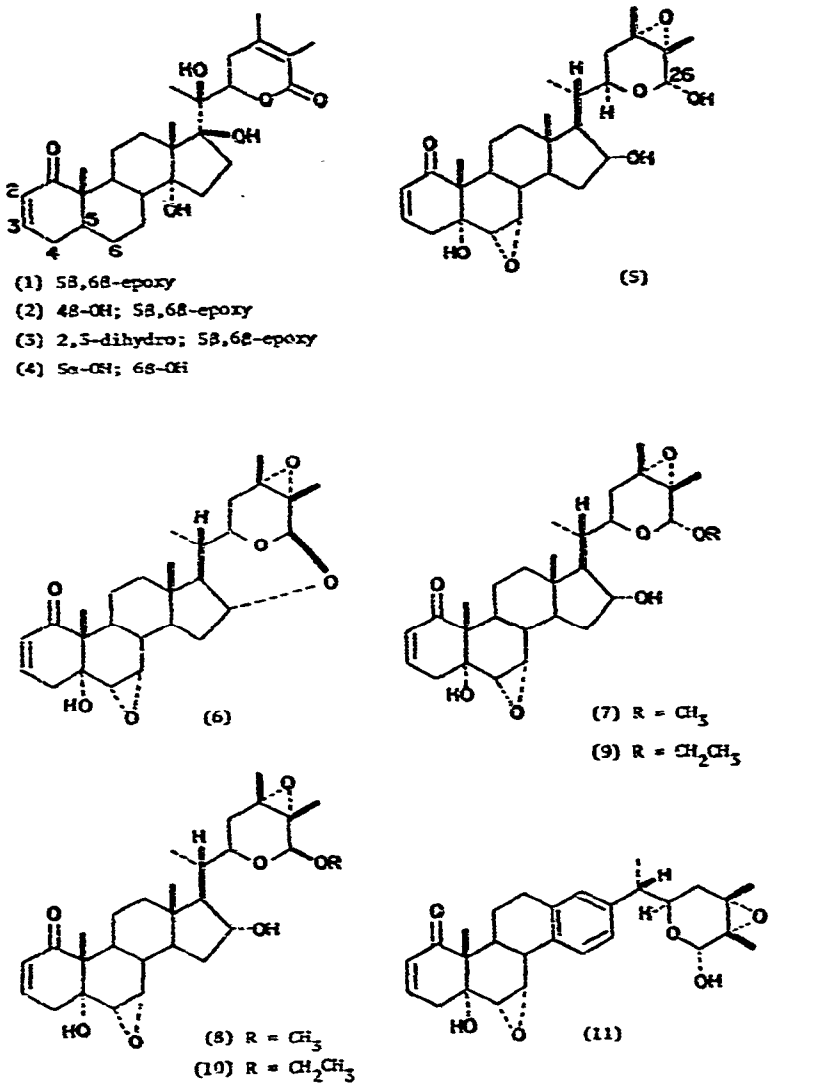


Fig. 1. Steroid structures.

(Merck, Darmstadt, G.F.R.) and the top half with an equal amount of basic alumina (Woelm, Eschwege, G.F.R.). This treatment was necessary for detection at 225 nm with air as a reference.

The extracts and compounds used were obtained and isolated from the leaves of *Physalis peruviana* L. and *Nicandra physaloides* (L.) Gaerth var. *albiflora*. These plants were raised from seeds at the experimental farm of the Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot, Israel.

RESULTS AND DISCUSSION

We used high-sensitivity detection at 225 nm as described by Hunter *et al.*[11], who reported a detection limit of 5 ng for the withanolides.

Owing to the high polarity of the compounds separated in this study and in order to keep the elution times to the minimum, highly polar solvents were used. With nicalbins, methylene chloride plus 6% of 2-propanol gave an excellent separation of all four components (Fig. 2). The same solvent system was therefore used for the analysis of the plant extracts (Figs. 3 and 4).

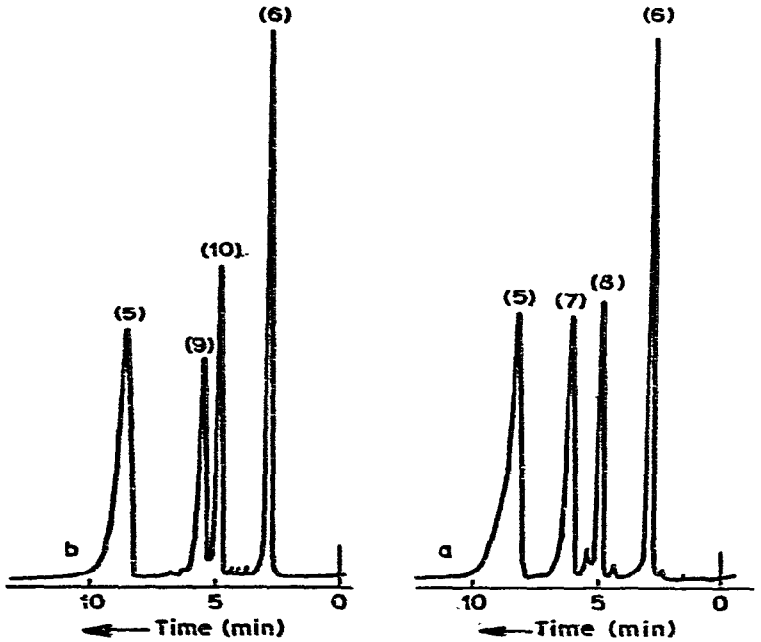
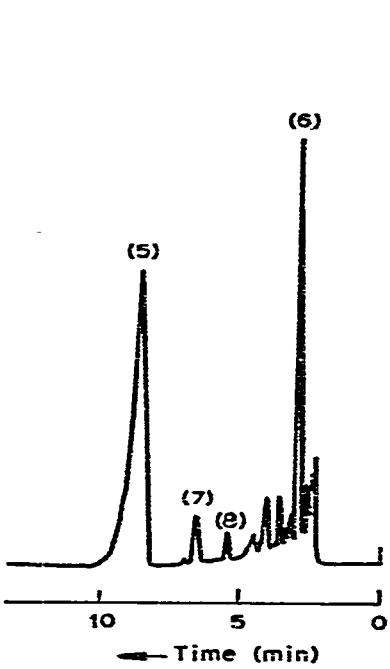


Fig. 2. (a) Chromatogram of standard mixture of nicalbin A (5), nicalbin A (26*R*)-methyl acetal (7), nicalbin A (26*S*)-methyl acetal (8) and nicalbin B (6). (b) Chromatogram of standard mixture of nicalbin A (5), nicalbin A (26*R*)-ethyl acetal (9), nicalbin A (26*S*)-ethyl acetal (10) and nicalbin B (6). Column: LiChrosorb SI-100, 5 μm. Solvent, methylene chloride–6% 2-propanol; flow-rate, 1.0 ml/min; detection, 225 nm.

The absence of Nic-1 (nicandrenone) was proved by the addition of a trace amount of the substance to the steroid mixture obtained by extraction of the leaves with ethanol (Fig. 5).

In the analysis of the constituents of *Physalis peruviana* we were faced with more polar compounds. Using the above mobile phase, withanolide E (1) and its 2,3-dihydroderivative (3) could not be separated* and 4β-hydroxywithanolide E (2) was eluted after a very long time. However, when acetonitrile–methylene chloride (1:1) was used, an excellent separation of steroidal lactones (1), (2) and (3) was

* At the time[7], the separation of this pair was extremely complicated.



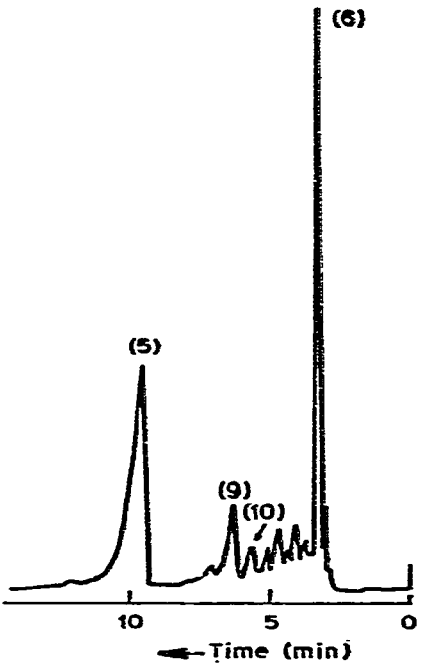


Fig. 3. Chromatogram of a crude methanolic extract obtained from leaves of *Nicandra physaloides* var. *albiflora*. For experimental conditions see Fig. 2.

Fig. 4. Chromatogram of a crude ethanolic extract obtained from leaves of *Nicandra physaloides* var. *albiflora*. For experimental conditions see Fig. 2.

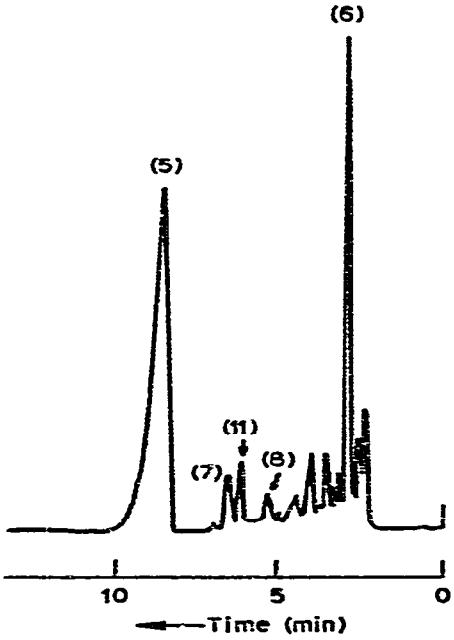


Fig. 5. Chromatogram obtained when a trace amount of Nic-1 (nicandrenone) (11) was added to the crude methanolic extract obtained from leaves of *Nicandra physaloides* var. *albiflora* (compare with Fig. 3).

achieved. This system was also applied to the crude plant extract (Fig. 6). Total separation, including withanolide S (4), was not possible in less then 30 min. The latter was eluted in a reasonable time when 2-propanol (5%) was added to the acetonitrile–methylene chloride (1:1) mixture.

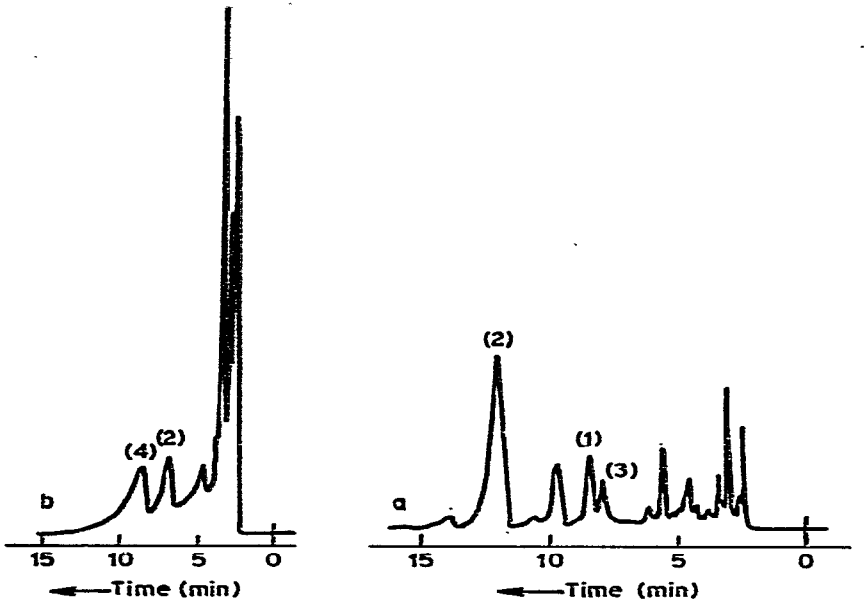


Fig. 6. (a) Chromatogram of a crude extract obtained from leaves of *Physalis peruviana*. Solvent: methylene chloride–acetonitrile (1:1). For other experimental conditions see Fig. 2. (b) Chromatogram of the same extract as above with methylene chloride–acetonitrile–2-propanol (47.5:47.5:5) as the solvent system.

CONCLUSIONS

Extracts and components of *Physalis peruviana* L. and *Nicandra physaloides* (L.) Gaerth var. *albiflora* provide a good demonstration of the advantages of HPLC for the analysis of solanaceous plants containing highly oxygenated steroids. The rapid and unequivocal separation of the epimeric hemiacetals, derived from nicalbin A during the alcoholic extraction procedure, and the clear distinction between withanolide E and its 2,3-dihydro derivative in the raw plant material, are noteworthy. This technique has obvious advantages in biosynthetic studies, in following the development of plants for optimal harvest time, and for selecting the preferred genotype of a given plant when one of the steroidal constituents is preferentially required.

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