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Note

Reversed-phase high-performance liquid chromatographic separation of steroidal thiazoles

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Steroidal thiazoles of the 17β-androstanyl-4-thiazole type are biologically and pharmaceutically important derivatives[1–3]. Members of this group show activities such as positive inotropic, cytotoxic and antimetabolic activity and inhibition activity *vs.* NaK-ATPase.

In order to determine the residues of untransformed thiazoles in biological material we developed a high-performance liquid chromatographic method that allows the separation of these compounds and their determination, using UV-monitored C_{18} reversed-phase chromatography with a polar eluent.

EXPERIMENTAL

Apparatus and chromatography

The liquid chromatograph consisted of a Milton Roy/LDC pump system support unit I, a stainless-steel column (250 × 4 mm I.D.) packed with Separon Si C_{18} (10 μm) (Laboratory Instruments, Prague, Czechoslovakia), a UVM-4 UV detector (Development Workshops of the Czechoslovak Academy of Science, Prague, Czechoslovakia) and a Knauer No. 63.00.00 loop injector.

Samples of 10 μl dissolved in dichloromethane were employed and eluted with methanol or methanol–water at a flow-rate 2 ml/min and 25°C. Based on the specific UV spectra of the steroidal thiazoles, 260 nm was chosen as a suitable wavelength for the detection of these compounds. In determining k' values, t_0 was determined as the retention time of the inflex point on the chromatogram, which was considered to represent the front of the chromatographic zone.

Materials and chemicals

Methanol was of pro analysis grade (Lachema, Brno, Czechoslovakia). The following compounds were prepared by literature methods: 17α-hydroxy-17β-[4-(2-amino-1,3-thiazolyl)]androst-4-en-3-one (I) and 17α-hydroxy-17β-[4-(2-amino-1,3-thiazolyl)]-androst-4-en-3,11-dione (II)[1]; 3β-acetoxy-17β-[4-(2-ethoxycarbonyl-1,3-thiazolyl)]-5-methyl-19-nor-5β-androst-9-ene (III)[2]; 3β-acetoxy-17β-[4-(2-ethoxycarbonyl-1,3-thiazolyl)]androst-5-ene (IV), 3β-acetoxy-17β-[4-(2-formamido-1,3-thiazolyl)]androst-5-ene (V)[3]; and 3β-hydroxy-17β-[4-(2-ethoxycarbonyl-1,3-thiazolyl)]-

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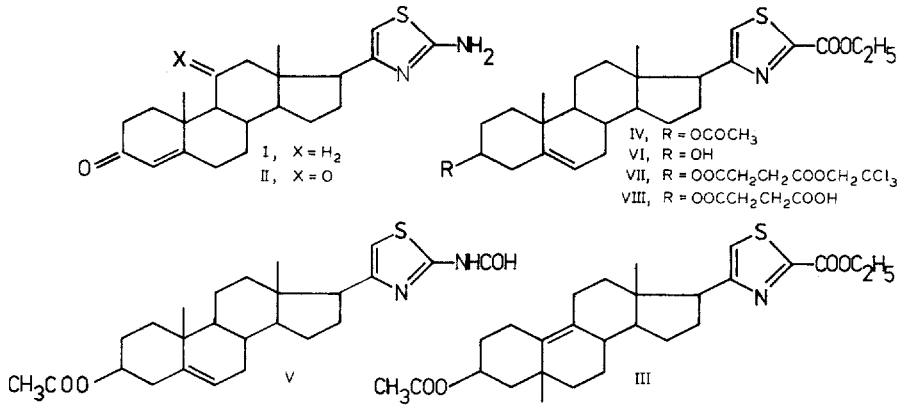

androst-5-ene (VI), 3β-hydroxy-17β-[4-(2-ethoxycarbonyl-1,3-thiazolyl)]androst-5-ene 3-[4-(2,2,2-trichloroethoxy)-4-oxobutanoate] (VII) and 3β-hydroxy-17β-[4-(2-ethoxycarbonyl-1,3-thiazolyl)]androst-5-ene 3-(3-carboxypropanoate) (VIII)[4].

RESULTS AND DISCUSSION

The described system allowed the analysis of the eight steroidal thiazoles I–VIII. Typical chromatograms obtained showed well resolved peaks. Over a period of several weeks the retention times of thiazoles varied slightly within the range ±3%. The wavelength chosen (260 nm) was characteristic of thiazoles and corresponds to the absorption of the thiazole ring[1,5]. The wavelength of detection enabled us to analyse mixtures of thiazoles in the presence of other steroidal derivatives. However, the analysis of all the thiazoles I–VIII in one chromatographic run was not the ex-

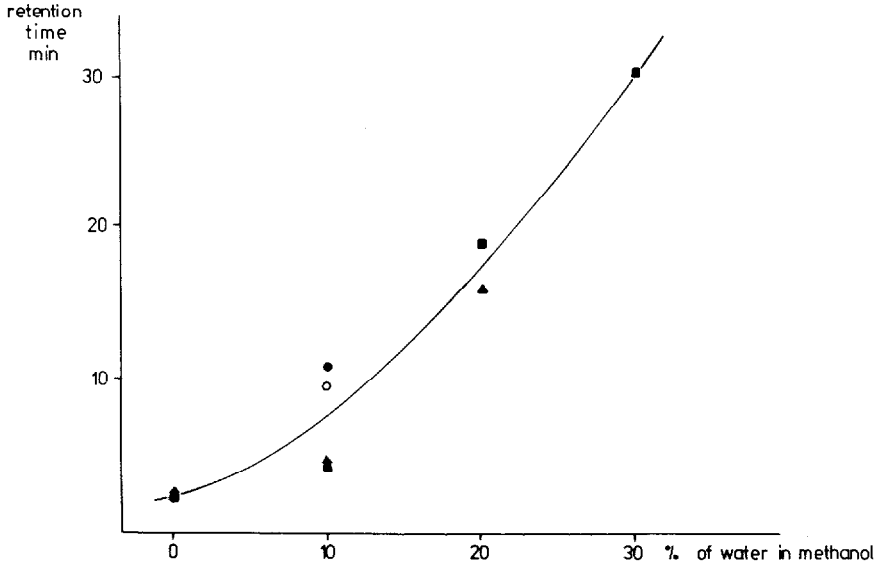

Fig. 1. Plot of retention time *versus* water content in the methanolic mobile phase. Compounds: ○, III; ●, IV; ▲, VI; ■, VIII.

TABLE I

RETENTION TIMES (t_R) AND CAPACITY FACTORS (k') WITH METHANOL

Solvent flow-rate, 2 ml/min; pressure, 4.92 MPa (700 p.s.i.); column packing, Separon Si C_{18} (10 μm). Samples were applied in dichloromethane solution.

Compound	*Retention time, t_R (min)*	*Capacity factor, k'*
I	2.67	0.76
II	1.87	0.24
III	2.80	0.85
IV	3.07	1.03
V	2.13	0.41
VI	2.24	0.48
VII	2.83	0.87
VIII	3.50	1.31

TABLE II

RETENTION TIMES (t_R) AND CAPACITY FACTORS (k') WITH METHANOL–WATER (9:1, v/v)

Solvent flow-rate, 2 ml/min; pressure, 7.03 MPa (1000 p.s.i.); column packing, Separon Si C_{18} (10 μm). Samples were applied in dichloromethane solution.

Compound	*Retention time, t_R (min)*	*Capacity factor, k'*
I	3.08	0.68
II	2.08	0.14
III	9.83	4.36
IV	11.25	5.14
V	8.67	3.73
VI	5.17	1.82
VII	16.17	7.82
VIII	4.75	1.59

TABLE III

RETENTION TIMES (t_R) AND CAPACITY FACTORS (k') WITH METHANOL–WATER (4:1, v/v)

Solvent flow-rate, 2 ml/min; pressure, 9.14 MPa (1300 p.s.i.); column packing, Separon Si C_{18} (10 μm). Samples were applied in dichloromethane solution.

*Compound**	*Retention time, t_R (min)*	*Capacity factor, k'*
I	16.08	7.58
II	4.00	1.13
VI	16.50	7.80
VIII	19.10	9.14

* The chromatographic conditions used are not suitable for the separation of the less polar thiazoles III, IV, V and VII.

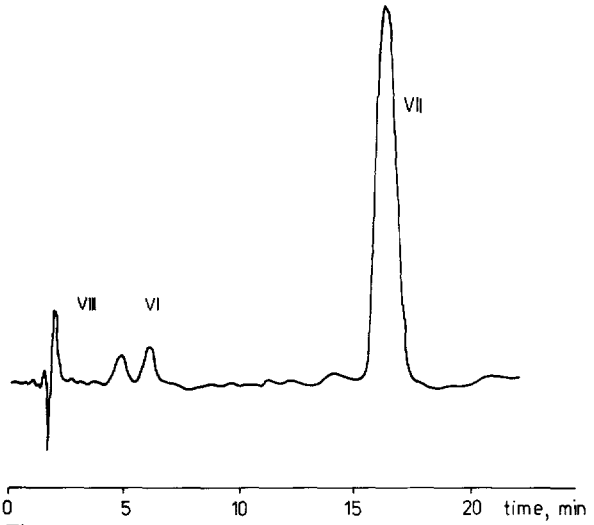

Fig. 2. Analysis in methanol–water (9:1, v/v) of the mixture after partial hydrolysis of VII in a protic medium.

clusive aim of this work, as there is no interest in applying them together in biological materials.

The three main eluent systems used for analysis were methanol, methanol–water (9:1, v/v) and methanol–water (4:1, v/v). Mobile phases with water contents higher than 20% appeared to be less suitable, as the steroidal thiazoles are not sufficiently soluble in such systems and the chromatograms show broad, tailing peaks with long retention times (Fig. 1).

The retention times and capacity factors of compounds I–VIII are summarized in Tables I–III. The best results were obtained with the methanol–water (9:1, v/v), all

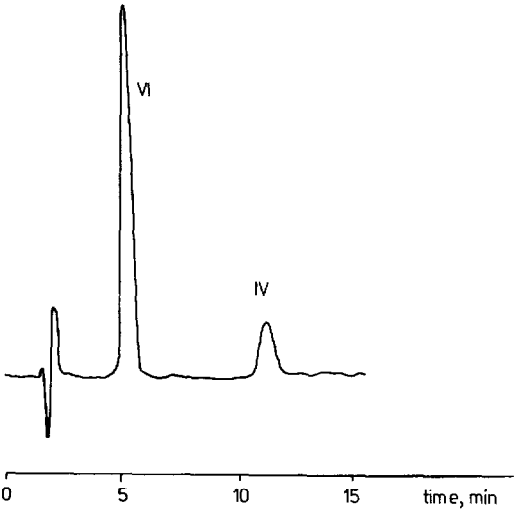

Fig. 3. Monitoring of the deacetylation of IV in a protic medium by high-performance liquid chromatography in methanol–water (9:1, v/v).

the thiazoles I–VIII being successfully separated in an isocratic run (Table II). With methanol–water (4:1, v/v) it was advisable to carry out the separations of the more polar thiazoles (I and II) from derivatives of the corticoid type, or alcohols, *e.g.*, IV. On the other hand, methanol allows a good separation of less polar thiazoles, *e.g.*, IV, VII and VIII. It should be noted that in groups of steroidal thiazoles with similar substitution and steroid skeleton, *e.g.*, I and II; III, IV, V and VI; or VI, VII and VIII, the retention times correspond to the polarity of the molecules, as expected (Figs. 2 and 3).

A study of the chromatographic behaviour of other analogues of steroidal cardiotonics and related heterocyclic derivatives is now in progress.

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REFERENCES

1 K. Takamura, C. Isono, S. Takaku and Y. Nitta, *Chem. Pharm. Bull.*, 11 (1963) 604.
2 P. Kočovský, P. Dražar, V. Pouzar and M. Havel, *Collect. Czech. Chem. Commun.*, 47 (1982) 108.
3 P. Dražar, F. Tureček and M. Havel, *Collect. Czech. Chem. Commun.*, 46 (1981) 2906.
4 P. Dražar, V. Pouzar, I. Černý and M. Havel, *Collect. Czech. Chem. Commun.*, 49 (1984) in press.
5 K. Takamura, C. Isono, S. Takama and Y. Nitta, *Chem. Pharm. Bull.*, 11 (1963) 613.