Journal of Chromatography, 481 (1989) 428–433
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 808

Note

Reversed-phase ion-pair high-performance liquid chromatographic separation and determination of tropane alkaloids in Chinese solanaceous plants

HE LI-YI, ZHANG GUAN-DE and TONG YU-YI
Institute of Materia Medica, Chinese Academy of Medical Sciences, 1 Xing Nong Tan Street, Beijing (China)
and
KAZUHIKO SAGARA, TOSHIYUKI OSHIMA* and TSUGUCHIKA YOSHIDA
Research Centre, Taisho Pharmaceutical Co., Ltd., 1-403, Yoshino-cho, Ohmiya-shi, Saitama 330 (Japan)
(First received March 28th, 1989; revised manuscript received July 10th, 1989)

Tropane alkaloids seem to be restricted to solanaceous plants. Hyoscyamine (I) and scopolamine (II) (Fig. 1) are tropane alkaloids and both have been used in clinical applications for a long time. Recently, two new tropane alkaloids, anisodamine [(−)-6-β-hydroxyhyoscyamine] (III) and anisodine (daturamine) (IV), were isolated in our institute from *Scopolia tangutica*. Both have distinct anticholinergic effects and can be used to cure various diseases[1–3].

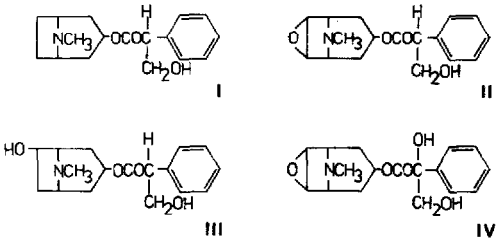

Fig. 1. Structures of the four tropane alkaloids. I = hyoscamine; II = scopolamine; III = anisodamine; IV = anisodine.

Scopolamine and hyoscyamine in crude drugs have been separated and determined by chromatographic techniques such as paper chromatography[4], thin-layer chromatography[5–7], gas chromatography[8,9] and high-performance liquid chromatography (HPLC)[10–13], but anisodine and anisodamine have been separated only by thin-layer chromatography and determined by spectrophotometry[5] or densitometry[6].

Recently, ion-pair HPLC has been applied to the determination of some natural compounds in plants[14–20]. We report here the application of reversed-phase ion-pair HPLC to the separation and determination of the above four tropane alkaloids in Chinese solanaceous plants.

0021-9673/89/$03.50

EXPERIMENTAL

Plant materials

Datura metel was collected in Shandong, Hunan and Guangdong, *Scopolia acutanglus* in Yunnan and *Scopolia lurida* and *Scopolia tangutica* in Qinghai and Tibet. These solanaceous plant samples were identified at the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China.

Apparatus

The method was developed using a Shimadzu LC-6A liquid chromatograph, equipped with a Shimadzu UV spectrophotometric detector, a stainless-steel column (150 × 4 mm I.D.) packed with chemically bonded ODS silica gel (TSK gel 120 A, 5 μm; Toyo Soda, Tokyo, Japan), a Shimadzu CTO-6A column oven and a Sic Chromatocorder 11.

Reagents

Anisodine hydrobromide, anisoamine hydrobromide and scopolamie hydrobromide were isolated from *Scopolia tangutica* and identified by means of IR, NMR and mass spectrometers at the Institute of Materia Medica. Atropine as a standard for hyoscyamine was purchased from BDH (Poole, U.K.). Sodium dodecyl sulphate and sodium phosphate (monobasic) were purchased from Wako (Osaka, Japan), sodium decyl sulphate and sodium octyl sulphate from Kanto (Tokyo, Japan), and benzylamine hydrochloride from Tokyo Kasei (Tokyo, Japan). Methanol of chromatographic grade was used. All other reagents were of analytical-reagent grade.

Internal standard solution. A 0.2 mg/ml solution of benzylamine hydrochloride in methanol was prepared.

Standard solutions. Stock standard solutions of anisodine, anisodamine, scopolamine and atropine were prepared in separate flasks by dissolution in methanol to a final concentration of 1 mg/ml as the free base. A 0.2-ml volume of each stock solution was transferred into a 10-ml volumetric flask, 2 ml of the internal standard solution were added and the mixture was diluted to 10 ml with the mobile phase to give a working standard solution, containing 20 μg/ml of the alkaloid. Working standard solutions of 40, 60, 80 and 100 μg/ml were prepared in the same manner.

HPLC conditions

The mobile phase was 1/15 *M* sodium phosphate solution (adjusted to pH 3.5 with phosphoric acid)–methanol (48:52) containing 17.5 m*M* sodium dodecyl sulphate. The column temperature was maitained at 35°C and the flow-rate was 1.0 ml/min. The eluted substances were detected by a UV detector at 210 nm.

Assay procedure

A powered plank sample (0.5 g) was macerated with 10.0 ml of chloroform and 0.15 ml of 25% ammonia solution in a 50-ml glass-stoppered flask and left to stand overnight. A 2-ml volume of the chloroform layer were transferred into a 5-ml volumetric flask and evaporated to dryness on a boiling water-bath. A 1-ml volume of internal standard solution was added to the residual substance and the mixture was diluted to 5 ml with the mobile phase. A 10-μl volume of this solution was injected into

the HPLC system. The concentrations of anisodine, anisodamine, scopolamine and hyoscyamine in solanaceous plants were calculated from the peak-area ratios with respect to the internal standard.

Calibration graphs and detection limits

All calibration graphs for anisodine, anisodamine, scopolamine and atropine were obtained over the concentration range 20–100 μg/ml. The corresponding

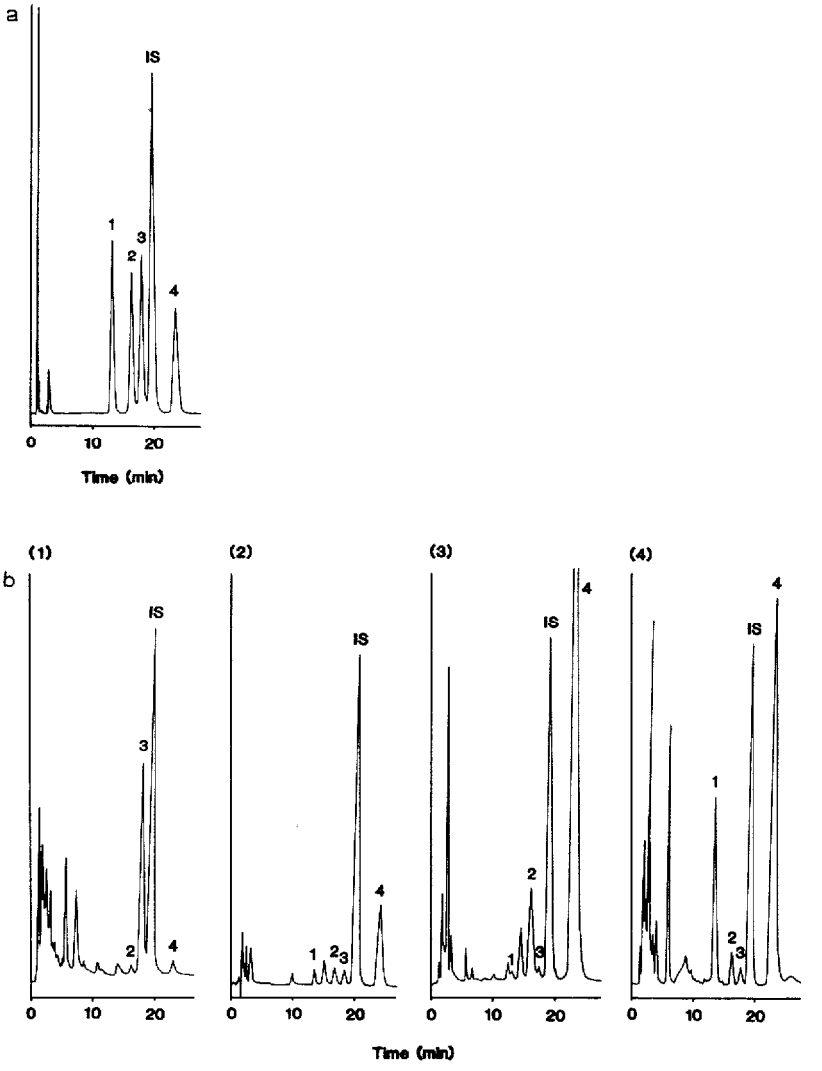

Fig. 2. Chromatograms of (a) standards and (b) plant samples. Plant samples: (1) *Datura metel* (Shandong); (2) *Scopolia acutangulus* (Yunnan:Zhongdian); (3) *S. lurida* (Qinghai:Haibei); (4) *S. tangutica* (Tibet:Ningjing). Peaks: 1 = anisodine; 2 = anisodamine; 3 = scopolamine; IS = internal standard (benzylamine hydrochloride); 4 = atropine. Mobile phase, 1/15 *M* sodium phosphate (pH 3.5)–methanol (48:52) containing 17.5 m*M* SDS; flow-rate, 1 ml/min; temperature, 35°C.

regression equations were $y = 0.95088x - 0.003335$ ($r = 0.999$), $y = 0.85741x - 0.004591$ ($r = 0.999$), $y = 0.90446x - 0.00335$ ($r = 0.999$), and $y = 0.80049x - 0.002557$ ($r = 0.999$) and the detection limits were 9.0, 7.5, 10.0 and 8.0 ng, respectively, at a signal-to-noise ratio of 3:1 for the peak heights.

RESULTS AND DISCUSSION

HPLC conditions

Various parameters such as counter ion, pH, concentration of organic modifier and ionic strength of the buffer on the ion-pair HPLC mobile phase were examined.

Sodium octyl sulphate (C_8), sodium decyl sulphate (C_{10}) and sodium dodecyl sulphate (C_{12}; SDS) were selected as counter ions. The pH of the mobile phase was examined over the range 2.0–6.0. The four tropane alkaloids were separated completely when SDS was used at a final concentration of 17.5 m*M* at pH 3.5. The methanol concentration in the mobile phase and that of sodium phosphate in the buffer were fixed at 52% and at 1/15 *M*, respectively. The column temperature was kept at 35°C to maintain constant retention times.

To enhance the reproducibility of the analytical results, about 30 compounds were tested for their suitability as internal standards under the above conditions. Benzylamine hydrochloride gave the best results. Fig. 2a shows a chromatogram of the four tropane alkaloids and the internal standard, which were well separated from each other, with an elution time of less than 25 min.

TABLE I

RECOVERY DATA FOR THE FOUR TROPANE ALKALOIDS

Compound	*Added (μg)*	*Recovery*		*Mean recovery (%)*	*Standard deviation (%)*	*Coefficient of variation (%)*
		μg	*%*			
Anisodine	0.0775	0.0768, 0.0756	99.10, 97.57	98.07	2.81	2.70
	0.1550	0.1571, 0.1598	101.37, 103.11			
	0.1550	0.1486, 0.1482	95.98, 95.63			
	0.3100	0.3025, 0.2919	97.59, 94.17			
Anisodamine	0.0762	0.0805	105.64	102.46	2.99	2.92
	0.1524	0.1630, 0.1534	106.97, 100.69			
	0.1524	0.1529, 0.1584	100.32, 103.95			
	0.3048	0.3105, 0.2981	101.87, 97.79			
Scopolamine	0.0789	0.0810, 0.0829	102.66, 105.06	106.35	1.93	1.82
	0.1577	0.1656, 0.1692	104.99, 107.25			
	0.1577	0.1697, 0.1713	107.62, 108.62			
	0.3154	0.3425, 0.3344	108.60, 106.02			
Hyoscyamine	0.0837	0.0795, 0.0788	94.98, 94.18	99.08	4.28	4.32
	0.1676	0.1750, 0.1661	104.39, 99.09			
	0.3352	0.3244, 0.3521	96.79, 105.05			

TABLE II

RESULTS OF ANALYSIS OF PLANT SAMPLES

Sample	*Locality*	*Part*[a]	*Content (%)*			
			Anisodine	*Anisodamine*	*Scopolamine*	*Hyoscyamine*
Datura metel	Shandong	fl	–	0.005	0.290	0.052
	Hunan: Changde	fl	–	0.027	0.560	0.073
	Guangdong	fl	–	0.018	0.270	0.057
Scopolia acutangulus	Yunnan: Lijiang	rt	–	0.020	0.046	0.470
	Yunnan: Lijiang	rt	–	0.012	0.003	0.130
	Yunnan: Zhongdian	rt	0.034	0.035	0.036	0.270
S. lurida	Qinghai: Haibei	rt	0.070	0.145	0.021	1.20
	Tibet: Zhamu	l	–	0.008	0.034	0.80
	Tibet: Yandong	st	–	0.005	0.054	0.47
S. tangutica	Qinghai	rt	0.006	0.058	–	0.200
	Tibet	rt	–	0.088	0.016	1.300
	Tibet: Ningjing	rt	0.200	0.036	0.023	0.690

[a] fl = Flowers; rt = root; st = stem.

Extraction conditions

The methods using the mobile phase[13] and basic chloroform[6,7] as the extraction solvent for scopolamine and hyoscyamine in solanaceous plants were compared. The two methods gave the same extraction efficiency from the crude drugs, but fewer impurities were extracted by the latter and the baseline was better than with the former. Therefore, the basic chloroform extraction method was chosen.

Of the powedered sample, 0.5 g was weighed accurately and three different amounts of the standard solution of four tropane alkaloids were added. The mixture was extracted and assayed according to the above procedure. The percentages of standards recovered were calculated by the internal standard method. Table I summarizes the recovery results and the statistical evaluation for each alkaloid.

Analytical results

Fig. 2b shows the chromatogram obtained on applying ion-pair HPLC to four kinds of solaneous plants.

Table II gives the analytical results. The concentration of scopolamine in *Datura metel* was more than five times higher than that of hyoscamine. In this study, we found that *Datura metel* contains a small amount of anisodamine.

CONCLUSIONS

Reversed-phase ion-pair HPLC was applied to the simultaneous determination of anisodine, anisodamine, scopolamine and hyoscyamine in Chinese solanaceous plants. This method is simple, reproducible and sensitive, and involves an isocratic HPLC system. This is the first report of the separation of the four tropane alkaloids anisodine, anisodamine, scopolamine and hyoscamine by HPLC. This quantitative method can be used for quality control and systematic research on solanaceous plants.

REFERENCES

1 Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, *Chin. Med. J.*, No. 2 (1975) 133 and No. 11 (1975) 795.
2 Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences, *Chin. Med. J.*, No. 53 (1973) 260.
3 Institute of Materia Medica, Chinese Academy of Medical Sciences, *Zhongcaoyao Tongxun*, 6 (1976) 10 and 7 (1976) 6.
4 A. Puech and J. L. Reffay, *Ann. Pharm. Fr.*, 27 (1969) 483.
5 B. L. Wu Chu, E. S. Kika, M. J. Soiomon and F. A. Crane, *J. Pharm. Sci.*, 58 (1969) 1073.
6 L. Y. He and Y. C. Chang, *Acta Pharm. Sin.*, 14 (1979) 421.
7 L. Y. He, *Zhongcaoyao*, 13 (1982) 13.
8 H. Mechler and H. W. Kohlenbach, *Planta Med.*, 33 (1978) 350.
9 M. Ylinder, T. Naaranlahti, S. Lapinjoki, A. Huhtikangas, M.-L. Salonen, L. K. Simola and M. Lounnasman, *Planta Med.*, (1986) 85.
10 S. Paphassarang and J. Raynand, *J. Chromatogr.*, 319 (1985) 412.
11 P. Duez, S. Chamart, M. Hanocq and L. Molle, *J. Chromatogr.*, 329 (1985) 415.
12 K.-H. Plank and K. G. Wagner, *Z. Naturforsch., Teil C*, 41 (1986) 391.
13 T. Oshima, K. Sagara, T. Yoshida, Y. Y. Tong, G. D. Zhang and Y. H. Chen, in preparation.
14 T. Misaki, K. Sagara, M. Ojima, S. Kakizawa, T. Oshima and H. Yoshizawa, *Chem. Pharm. Bull.*, 30 (1982) 354.
15 K. Sagara, T. Oshima and T. Misaki, *Chem. Pharm. Bull.*, 31 (1983) 2359.
16 K. Sagara, Y. Ito, T. Oshima, T. Misaki and H. Murayama, *J. Chromatogr.*, 328 (1985) 289.
17 K. Sagara, Y. Ito, M. Ojima, T. Oshima, K. Suto, T. Misaki and H. Itokawa, *Chem. Pharm. Bull.*, 33 (1985) 5369.
18 K. Sagara, T. Oshima and T. Yoshida, *J. Chromatogr.*, 403 (1987) 253.
19 K. Sagara, T. Oshima, S. Sakamoto and T. Yoshida, *J. Chromatogr.*, 388 (1987) 448.
20 K. Sagara, T. Oshima and T. Yoshida, *J. Chromatogr.*, 409 (1987) 365.