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High-Performance Liquid Chromatographic Determination of Organic Substances by Metal Chelate Derivatization. III.[1] Analysis of Ephedra Bases

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High-performance liquid chromatographic (HPLC) analysis of ephedrine, pseudoephedrine, norephedrine and pseudonorephedrine in some Ephedra species was carried out. Color reaction of these bases by the formation of Ni(II) dithiocarbamate chelates facilitated microdetermination of these based at levels of less than 1 ng. This method was applied to the analysis of these bases in some Ephedra species. The present method is so sensitive that analysis of these bases in Pinelliae Tuber was also possible, and peaks corresponding to pseudoephedrine, norephedrine and pseudonorephedrine appeared on the chromatograms along with the peak of ephedrine.

Keywords——ephedrine; pseudoephedrine; norephedrine; pseudonorephedrine; Ephedra species; Ephedrae Herba; Pinelliae Tuber; Ni(II) dithiocarbamate; HPLC

Determination of ephedra alkaloids in Ephedrae Herba (麻黄) has been carried out by thin layer chromatography (TLC),[2] gas-liquid chomatography (GLC),[3] carbon-13 nuclear magnetic resonance (^{13}C-NMR)[4] and the titration method stipulated in JP X. High-performance liquid chromatography (HPLC) has also been utilized in recent years.[5] Normal phase chromatography on silica gel packings, however, tends to cause tailing, and resolution is not satisfactory.[5a] Reverse phase ion-pair chromatography using sodium lauryl sulfate gives better results.[5b] This method, however, requires a high column temperature. Another problem in the microdetermination of these bases is that they have only weak absorption in the ultraviolet (UV) range. A rapid and quantitative color reaction is, therefore, desirable. In our previous reports,[1,6] HPLC determination of aliphatic primary and secondary amines including stimulant drugs by the formation of Ni(II) dithiocarbamate chelates was described. The present report deals with the determination of several ephedra bases in plant materials.

Experimental

Apparatus——The HPLC apparatus used is identical with that described in our previous report.[1]

Plant Materials——Several Ephedra species were collected at the Botanical Garden of Gifu College of Pharmacy. Commercial Ephedrae Herba and Pinelliae Tuber (半夏) were also used.

Standard Samples——*l*-Ephedrine hydrochloride and *dl*-norephedrine hydrochloride were obtained commercially. *d*-Pseudoephedrine hydrochloride (mp 182 °C) was synthesized from *l*-ephedrine by acetylation of *l*-ephedrine, followed by hydrolysis with hydrochloric acid.[7] *dl*-Pseudonorephedrine hydrochloride (mp 169 °C) was also synthesized by a similar procedure[8] from *dl*-norephedrine. These salts, twice recrystallized from acetone, each showed a single peak on HPLC chromatograms, and no residual starting material, *l*-ephedrine or *dl*-norephedrine, was detected.

Extraction of Plant Materials——Powdered ephedra samples (200 mg) were extracted with three 15 cm^3 portions

of 0.2 M sulfuric acid as described elsewhere.[9] Extraction of Pinellia Tuber was carried out by a similar procedure with a larger sample amount (5 g). Then the sulfuric acid solution was diluted to 50 cm^3 with water.

Derivatization Method——The color reaction was carried out by a procedure similar to that described in our previous report[1] with some modifications. A 0.1 M solutions of nickel(II) chloride in 1 M trisodium citrate (10 cm^3) was added to an aliquot of dilute sulfuric acid solution of plant extract (10 cm^3). The pH was adjusted to 12.5 and higher with 1 M sodium hydroxide, then 5% carbon disulfide in chloroform–ethyl acetate = 1 : 1 (8 cm^3) was added. The mixture was shaken well for 3 min in a small separating funnel, and thus color reaction and extraction were carried out. The aqueous layer was extracted with 2 cm^3 of chloroform : ethyl acetate = 1 : 1 once more, and then the volume of the organic layer was made up to exactly 10 cm^3. Next, Ni(II) dibutyldithiocarbamate solution (NiX_2) was added to an aliquot of the above solution so that the content of X represented more than 100-fold excess in molar ratio. The color reaction can be represented as follows.

$$R_1R_2NH + CS_2 + OH^- + 1/2Ni^{2+} \rightarrow 1/2(R_1R_2NCSS)_2Ni(=NiA_2) + H_2O \quad (1)$$

$$NiA_2 + NiX_2 \rightleftharpoons 2NiAX \quad (2)$$

The mixture was kept standing for 2 h, and then subjected to HPLC.

Results and Discussion

Figure 1(a) shows a chromatogram of a standard mixture of *l*-ephedrine, *d*-pseudoephedrine, *dl*-norephedrine and *dl*-pseudonorephedrine. In this sample, NiX_2 was added so that the content of X was larger than 100-fold excess over the sum of the four bases in molar amount. Thus, more than 99% of these bases should be converted into the ternary complex (NiAX, NiBX, *etc.*). After the large peak of NiX_2, four peaks corresponding to individual amines appeared on the chromatograms. No peak appeared corresponding to the retention times of binary complexes (NiA_2, NiB_2, *etc.*).

Calibration curves for the four bases were obtained. These curves were linear up to about 5 μg. The detection limits were 0.5 ng and 1.5 ng for *l*-ephedrine and *dl*-pseudonorephedrine, respectively, under the conditions shown in Fig. 1(a). Determination of larger amounts (> 5 μg) of these bases was sometimes interfered with by the presence of the very large amount of

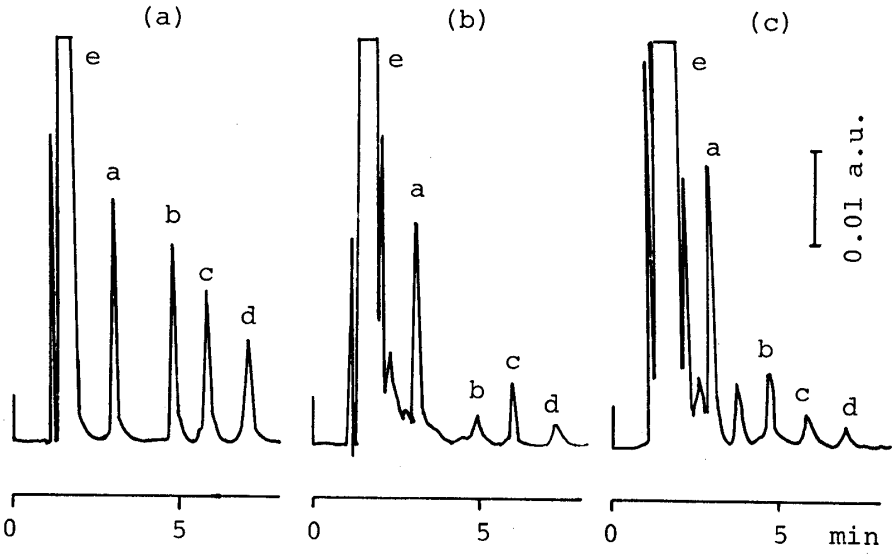

Fig. 1. Chromatograms of Ephedra Bases

Column: Polygosil 60-5 (5 μm), 4 mm × 25 cm.
Eluent: hexane–isopropyl acetate = 100 : 15 (water-saturated) at 2.0 ml/min.
Detection: UV 325 nm (full scale 0.1 a.u.).
(a) Standard sample (80 ng *l*-ephedrine, 80 ng *d*-pseudoephedrine, 75 ng *dl*-norephedrine and 75 ng *dl*-pseudonorephedrine in each injection); (b) *E. sinica*; (c) Pinelliae Tuber.
Sample size: (a) 5 μl; (b) 50 μl; (c) 50 μl.
a, *l*-ephedrine; b, *dl*-norephedrine; c, *d*-pseudoephedrine; d, *dl*-pseudonorephedrine; e, diethylamine (peak of excess binary complex MX_2).

TABLE I. Contents of Ephedra Alkaloids in Various Ephedra Species

Species	Ephedrine (%)	Pseudoephedrine (%)	Norephedrine (%)	Pseudonorephedrine (%)
E. sinica STAPF	0.033	0.075	0.0076	0.017
E. distachya L.	0.19	0.018	0.0022	0.0017
E. regeliana FLORIN	0.054	0.10	0.0012	0.0015
E. intermedia SCHRENK *et* C. A. MEY	0.058	0.72	0.0005	0.0068
E. tweediana C. A. MEY	0.0028	0.011	0.0005	0.0003
E. equisetina BGE	0.23	0.21	0.0042	0.010
E. procera C. A. MEY	0.06	0.19	0.0004	0.0012
E. ciliata C. A. MEY	—	—	—	—
E. gerardiana WALL	0.46	0.07	0.0030	0.0072
Ma-Huang (Commercial)	0.50	0.20	0.0029	0.0042

These values corresponded to contents of bases in samples of various dried Ephedra species.

NiX_2 which overloaded the column. Since the Ni(II) dithiocarbamate complexes have very strong absorption ($\log \varepsilon \fallingdotseq 4.5$ at 325 nm)[9] compared with those of non-derived ephedra bases ($\log \varepsilon = 2.31$ at 259 nm),[10] the present color reaction provides an increase in sensitivity of about 200-fold.

The present method was applied to the determination of these bases in several Ephedra species. An example of the chromatograms and the analytical data are shown in Fig. 1(b) and Table I, respectively. These four bases were all contained in all the Ephedra species except *E. ciliata*, though the contents of norephedrine and pseudonorephedrine were very small. The ratio of *l*-ephedrine to *d*-pseudoephedrine differs from the results of other authors.[3] The discrepancy may be due to differences in environmental factors.

Since the present method can detect very small amounts of ephedra bases other than tertiary amines, an attempt was made to detect these bases in Pinelliae Tuber. The presence of a very small amount of *l*-ephedrine in this crude drug has been reported recently,[11] although the presence of other ephedra bases has not been confirmed. Fig. 1(c) shows an example of the chromatograms. *l*-Ephedrine was detected in all four samples tested. Several other peaks appeared, and measurements of the retention times revealed that these peaks have retention times identical with those of pseudoephedrine, norephedrine and pseudonorephedrine within 0.02 min. These three peaks were recognized in three samples among the four samples tested. Addition of *d*-pseudoephedrine, *dl*-norephedrine and *dl*-pseudonorephedrine to the plant extract increased the heights of these three peaks. Thus, it seems probable that these three bases are also contained in Pinelliae Tuber in very small amounts. The content of *l*-ephedrine in the sample shown in Fig. 1(c) amounted to only 0.0002%. Since the present color reaction has very high sensitivity, very small amounts of these bases in other plant materials should be detectable by HPLC.

References and Notes

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