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Qualitative and Quantitative Analysis of Ephedra Alkaloids in Ephedrae Herba by Carbon-13 Nuclear Magnetic Resonance

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A ^{13}C-nuclear magnetic resonance procedure is described for the rapid qualitative and quantitative analysis of ephedra alkaloids (I and II). This technique has been applied to the analysis of basic fractions from various ephedrae herba, giving results comparable to those obtained by gas–liquid chromatography technique, but with many advantages.

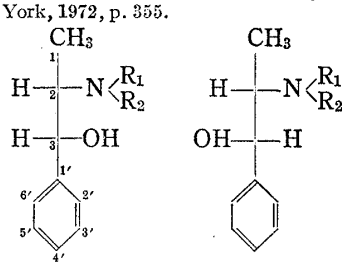
Keywords——qualitative analysis; quantitative analysis; ephedra alkaloids; ephedrine; Ephedraceae; carbon-13 NMR

The oriental crude drug, ephedrae herba, "Ma-Huang," contains sympathomimetic amines, *l*-ephedrine (I), *d*-pseudoephedrine (II), and some other related alkaloids.[2] To evaluate the crude drug, titration of the total alkaloids is generally used.[3] However, due to some differences of physiological activities among these alkaloids,[2] separative analysis of each alkaloids should be necessary. Several attempts have been reported for the separative

Table I. ^{13}C Chemical Shifts Ephedra Alkaloids (HCl Salts) in D_2O Solution (δ_C from TMS)[a]

Carbon	*l*-Ephedrine (I)	*d*-Pseudoephedrine (II)	*l*-Methylephedrine (III)	*l*-norephedrine[b] (VI)
C-1	10.6	12.8	8.5	13.3
C-2	60.8	60.5	67.4	53.3
C-3	72.1	75.5	71.5	73.7
N-Me	31.7	30.9	41.1 42.8	—
C-1′	139.4	140.5	140.3	139.4
C-2′,6′	126.9	127.8	126.8	127.1
C-3′,5′	129.6	129.8	129.8	129.7
C-4′	129.2	129.8	129.3	129.4

a) Calculated from internal dioxane (δ_C=67.4).
b) L.F. Johnson and W.C. Jancowsky, "Carbon-13 NMR Spectra," Wiley-Interscience, New York, 1972, p. 355.



I: R_1=H, R_2=Me II: R_1=H, R_2=Me
III: R_1=R_2=Me
VI: R_1=R_2=H

1) Location: *1-2-3, Kasumi, Hiroshima-shi, 734, Japan.*
2) L. Reti, "The Alkaloids," Vol. III, ed. by R.H.F. Manske and H.L. Holmes, Academic press, New York, 1953, p. 339.
3) Japanese Pharmacopoeia ed. IX, Part II, p. 1163.

analysis of these alkaloids, including our method based on gas chromatography of oxazolidine derivatives of these bases.[4] The present report deals with more rapid and reliable method for separative analysis of I and II in ephedrae herba by ^{13}C-nuclear magnetic resonance (abbreviated CMR) spectroscopy.

Two main alkaloids in Ephedrae Herba are I (up to *ca.* 1%), and II (up to *ca.* 0.6%). The minor ones are *l*-methylephedrine (III) (less than 0.04%), and some others trace quantity of less than 0.01%.[4,5] CMR spectra of these compounds (HCl-salt) were recorded in D_2O solutions, and chemical shift values are listed in Table I. As each compound shows characteristic chemical shift pattern on CMR spectrum, identification (qualitative analysis) of the compounds can be readily achieved even in the mixed form. In the mixture of I and II, five sets of corresponding carbon resonance peaks (C-1′, C-2′,6′, C-3, N–Me, C-1) were clearly separated, though the other resonance peaks were overlapped.

To decide the repetition time which can be used for quantitative study of Ephedra alkaloids, longitudinal relaxation times (T_1) of I and II were measured by inversion recovery technique (Table II). It was found that T_1 values are less than 3.2 sec, except for C-1′ resonances of both compounds which have no bonded protons. Accordingly, when flip angle of

Table II. Spin-Lattice Relaxation Time (T_1) in sec of Ephedra Alkaloids (HCl Salts)[a]

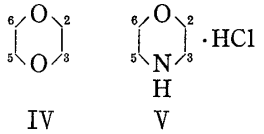
Carbon	*l*-Ephedrine (I)	*d*-Pseudoephedrine (II)
C-1	1.9	2.2
C-2	1.9	1.9
C-3	1.8	2.0
N-Me	3.2	2.0
C-1′	>6	>6
C-2′,6′	2.0	1.9
C-3′,5′	2.1	—[b]
C-4′	1.5	—[b]

a) Measured at 25° in D_2O solutions at the concentrations of 100 mg/ml (0.5 M) by inversion recovery method. Reproducibility of the measured T_1 values was ±10%.
b) Not measured due to overlapping of the signals.

Table III. Spin-Lattice Relaxation Times (T_1/sec) of Internal Standard Compounds[a]

Compounds	Carbons	Chemical shifts (δ)	T_1 (Concentrations/M)		
Dioxane (IV)	C-2,3,5,6	67.4	3.4 (0.23)		
Morpholine HCl salt (V)	C-2,6	64.5	4.5 (0.69)	3.7 (1.0)	3.3 (1.7)
	C-3,5	44.2	4.5 (0.69)	3.8 (1.0)	3.3 (1.7)

a) Measured at 25° in D_2O solutions by progressive saturation methods (successive single 90° pulsed). Reproducibility of the measured T_1 values was ±5%.



IV V

4) K. Yamasaki, K. Fujita, M. Sakamoto, K. Okada, M. Yoshida, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **22**, 2898 (1974), and the references cited therein.
5) Unpublished results obtained by the method in ref. 4).

90° is adopted, repetition time of 13 (=4×3.2) sec is enough to reach the "constant condition" for quantitative analysis of both compounds.[6,7] Blunt and Munro reported another approach to set the condition for quantitative analysis of mixtures of carbohydrates.[8] However, their conditions (45° flip angle) hold for only equal or nearly equal concentrations of the samples, which is not prospecting method for the analysis of ephedra bases in ephedrae herba.

Internal Standard Compound

Dioxane (IV) and morpholine hydrochloride (V) were chosen as internal standard. The relaxation times (T_1) of both compounds were measured by inversion recovery and/or progressive saturation method (Table III). From the results obtained, both compounds were suitable for internal standard if the repetition time of more than 15 sec for IV, and 20 sec for V are adopted. For recovery of the sample, IV is better than V, but the resonance peak of IV would overlap to C-2 of III, though the relative content of III is usually negligible for practical analysis of the crude drug. Two resonance peaks of V appear at 'clear' regions in CMR spectra of ephedra alkaloids, so the both peaks can be used for standards, to increase the accuracy.

Working Curves

Theoretically, peak intensity (M) should be corresponded to peak areas. In the present work, however, peak height is used instead of peak areas due to instrumental restriction and experimental simplification. As a check, the peak heights of a sample of I with IV were plotted against the peak areas. A straight line curve passing through the origin was obtained except the resonance peak of C-1′ (Fig. 1). Then, CMR spectra of I with internal standards IV (in 2% D_2O solution) were recorded at several different concentrations (20—100 mg/ml). The peak height ratios of the C-1, C-3, N–Me, C-2′, 6′, and C-4′ of I to the standard peak

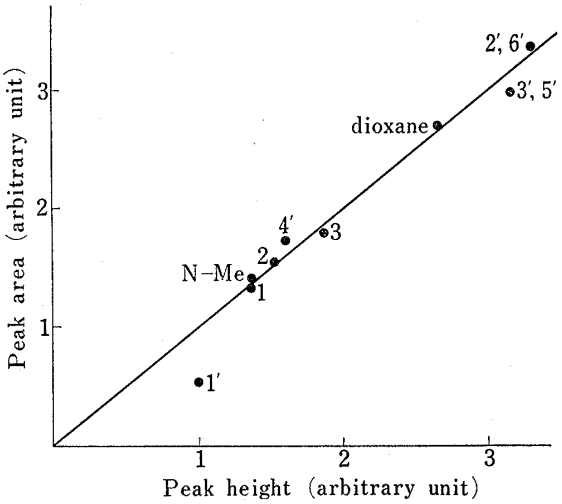


Fig. 1. Correlation of Peak Area to Peak Height

Sample: I.
Standard: IV.

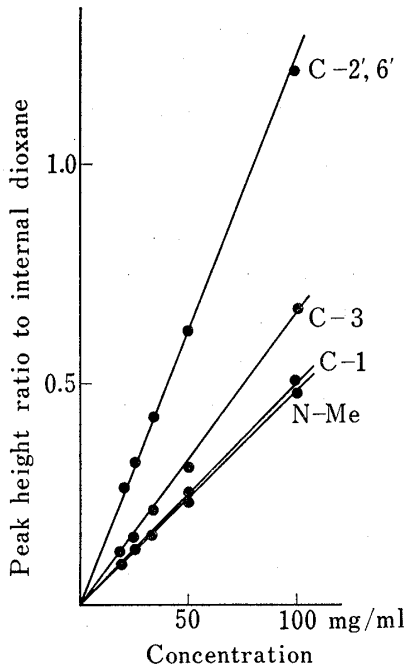


Fig. 2. Calibration Curves of Ephedrine Hydrochloride[a)]

a) Measured in 2% solution of dioxane in D_2O.

6) G.C. Levy and G.L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, 1972.
7) R. Freeman and H.D.W. Hill, *J. Magn. Reson.*, **4**, 366 (1971).
8) J.W. Blunt and M.H.G. Munro, *Aust. J. Chem.*, **29**, 975 (1976).

of IV, were plotted against the concentration of I to obtain straight lines passing through the origin ($y=ax$), although the slopes (a) for each carbon resonances are different due to nuclear overhauser effect (NOE) (Fig. 2). In the same manner, four sets of curves were obtained for samples I or II to standards IV or V. The slope (a value) were listed in Table IV.

Table IV. The Slopes ('a') of Calibration Curves[a]

Standard compounds	Carbons	Samples C-1	N-Me	C-3	C-2′,6′
		Ephedrine HCl (I)			
Dioxane (IV)	C-2,3,5,6	0.103	0.101	0.135	0.257
Morpholine HCl (V)	C-2,6	0.337	0.307	0.364	0.790
	C-3,5	0.415	0.378	0.448	0.975
		Pseudoephedrine HCl (II)			
Dioxane (IV)	C-2,3,5,6	0.101	0.100	0.131	0.247
Morpholine HCl (V)	C-2,6	0.341	0.317	0.413	0.863
	C-3,5	0.380	0.353	0.460	0.959

a) 'a' Value is defined by the equation, $y=ax$, where x is weight ratio of sample to standard, y is peak height ratio of sample to standard.

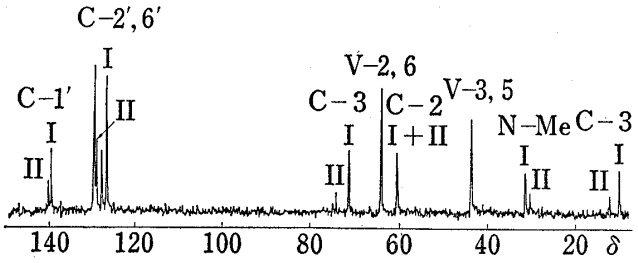


Fig. 3. ^{13}C NMR Spectrum of Basic Fraction (HCl Salt) obtained from Commercial Ephedrae Herba (15 g) with Morpholine (V)

Analysis of some Ephedrae Herba

The basic fraction obtained from *ca.* 15 g of ephedrae herba were analyzed directly by CMR in D_2O with internal standard IV or V (see experimental). CMR spectrum of one of these examples is shown in Fig. 3. Four peak heights associated with I were compared with standard peaks of V, and the ratios were calculated. These values were devided by the 'a' values in Table IV and averaged to obtain the quantity of I. In the same manner, the quantity of II was also obtained. Minor alkaloids such as III could not be detected. Although the deviations of each 'a' values are estimated to exceed more than 10%, averaged value of 4 or 8 data minimize them to less than 5%.

Comparison to GLC Method

The analytical results for several Ephedrae Herba were compared to the results obtained by gas-liquid chromatography (GLC) (Table V).[4,5]

Both methods gave essentially the same values. As far as sensitivity is concerned, GLC is far better than CMR. By GLC, minor alkaloids such as III and norephedrine could

Table V. Contents of Ephedra Alkaloids (I and II) in Ephedrae Herba, Analyzed by Two Different Methods, CMR and GLC[5] (% of Dry Weight)

Samples[a]	Ephedrine (I) CMR	(GLC)	Pseudoephedrine (II) CMR	(GLC)
1	0.66±0.02	(0.64)	0.27±0.02	(0.27)
2	0.51±0.01	(0.50)	0.19±0.01	(0.22)
3	0.17±0.03	(0.17)	0.32±0.03	(0.33)
4	0.93±0.01	(0.94)	0.14±0.03	(0.14)

a) 1-3, Obtained from Hiroshima Market. 4, *Ephedra nebrodensis* var. *procera* (Baluchistan).

be detected in the crude drug, whereas by CMR, due to the restriction of computer-limited dynamic range in longitudinal axis, these minor alkaloids could not be detected. However, CMR method has great advantages over GLC in some points. With respect to qualitative identification of each compound, only one peak is used in GLC, whereas in CMR, seven or eight sharp specific peaks for each compound are used, resulting unambiguous confirmation. As for time consumption, derivative formation which requires 6 hr in GLC method, is not necessary in CMR method. Half an hour or at most one hour measurement of basic fraction (HCl-salt) from the crude drugs (*ca.* 15 g) is enough to obtain sufficient signal to noise (S/N) ratios for calculations of the quantity. After CMR measurement, recovery of the samples (*e.g.* for pharmacological test) is indeed possible and easy especially with the standard IV.

Experimental

Extraction——Dried sample of Ephedra Herba (20 g) was extracted with boiling H_2O for 1.5 hr (600 ml×2). After filtration, the combined extract was concentrated to *ca.* 50 ml. To this solution, dil. HCl was added to pH 2—3. After removing the acidic and neutral substances by extraction with ether (80 ml×3), the acidic aqueous solution was made alkaline with K_2CO_3 to pH 11, and then extracted with ether (80 ml×3). The ether extract was washed with a small amount of 5% K_2CO_3 in aqueous solution, and evaporated *in vacuo* below 30°. Evaporation was repeated by adding a small amount of benzene to dryness. The residue (basic fraction) was dried in desiccator over Si gel for overnight at room temperature. 3/4 Portion of the residue was neutralized by HCl and subjected to CMR analysis and the other portion was analyzed by GLC.[4]

CMR Measurements——The basic fraction from 15 g (20 g×3/4) of ephedrae herba was dissolved in 1 ml of D_2O and 85.7 mg of V was added. CMR spectrum of the sample was recorded on a JEOL PFT 100 spectrometer equipped with EC-6 computer at 25.15 MHz in 10 mm spinning tube at 25°. FT-measuring conditions were as follows, data points, 8192; spectral range, 4 KHz; pulse flip angle, 90°; repetition time, 20 sec; numbers of transients, 100—200; acquisition time, 1.1 sec; computer limited resolution, ±0.04 ppm; window function, exponental-8.

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