[Chem. Pharm. Bull. 28(10)2900—2904(1980)]

Antiinflammatory Principle of *Ephedra* Herbs[1]

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(Received March 18, 1980)

Since the traditional usage of an Oriental medicine "maō", consisting of *Ephedra* herbs, suggests that it may possess antiinflammatory activity, a crude drug preparation, the aerial part of *E. intermedia*, was subjected to bioassay for antiinflammatory activity by two methods. The methanol extract was found to be active. The extract was fractionated in monitoring the antiinflammatory activity, resulting in the isolation of (+)-pseudoephedrine as the active principle. The antiinflammatory effects of ephedrine analogs were determined by four assay methods; pseudoephedrine exhibited the strongest activity in the Whittle method, the carrageenin paw edema method in mice, and the fertile egg method.

Keywords——antiinflammatory activity; *Ephedra* herbs; Ephedraceae; (−)-ephedrine; (+)-pseudoephedrine

The crude drug "maō", prepared from the aerial part of certain species of *Ephedra* plants (Ephedraceae), is famous for containing alkaloids of the ephedrine series[3] having sympathomimetic and anti-allergic activity. Although a number of other substances have been isolated from *Ephedra* plants,[4] none of them is known to be physiologically active, except for flavonoids having vitamin P activity.[5] The perspiratory, anti-tussive and anti-allergic effects of the crude drug can be rationalized in terms of the effects of its main alkaloid, (−)-ephedrine. Further examination of prescriptions commonly utilized in Oriental medicine and involving "maō" as a component revealed that they are frequently administered in anticipation of antiinflammatory action as one of the therapeutic effects. Since no antiinflammatory principles have yet been found in the crude drug, the present work was initiated.

The antiinflammatory action of a commercially available preparation, an *Ephedra intermedia* herb from China, was investigated by two different assay procedures, the Whittle method and the fertile egg method, and it was found that the methanol extract reduced the increase of vascular permeability induced by acetic acid in mice (39% inhibition ($p<0.05$) at a dose of 5 g (crude drug equivalent)/kg) and inhibited granulation tissue formation of the chorioallantoic membrane of the chick embryo (37% inhibition at a dose of 2.5 mg (crude drug equivalent)/disc). At this stage, it seemed probable that these physiological actions were mediated by the main alkaloid, (−)-ephedrine. The content of (−)-ephedrine in a preparation of good quality is said to reach 1% of the crude drug. (−)-Ephedrine was thus tested for antiinflammatory activity by means of the Whittle method, but exhibited no significant activity even at a dose (50 mg/kg) equivalent to 5 g of the crude drug, taking the ephedrine content to be 1%. It was thus concluded that the antiinflammatory effects shown by the crude drug were not associated with (−)-ephedrine. The extract was then fractionated, monitoring in terms of antiinflammatory activity determined by the Whittle method, as shown in Chart 1.

The methanol extract was extracted with ethanol, and the active ethanol soluble fraction was divided into acidic, neutral and basic portions. Only the basic portion was active, and

1) Studies on the constituents of *Ephedra*. VI. This paper also constitutes Part XV in the series on the validity of the Oriental medicines.
2) Location: *Aoba-yama, Sendai.*
3) S.W. Pelletier, "Chemistry of the Alkaloids," Van Nostrand Reinhold Co., New York, 1970, pp. 24—25.
4) *cf.* M. Tamada, K. Endo, and H. Hikino, *Planta medica*, **34**, 291 (1978).
5) H. Friedrich and H. Wiedemeyer, *Planta medica*, **30**, 163 (1976).

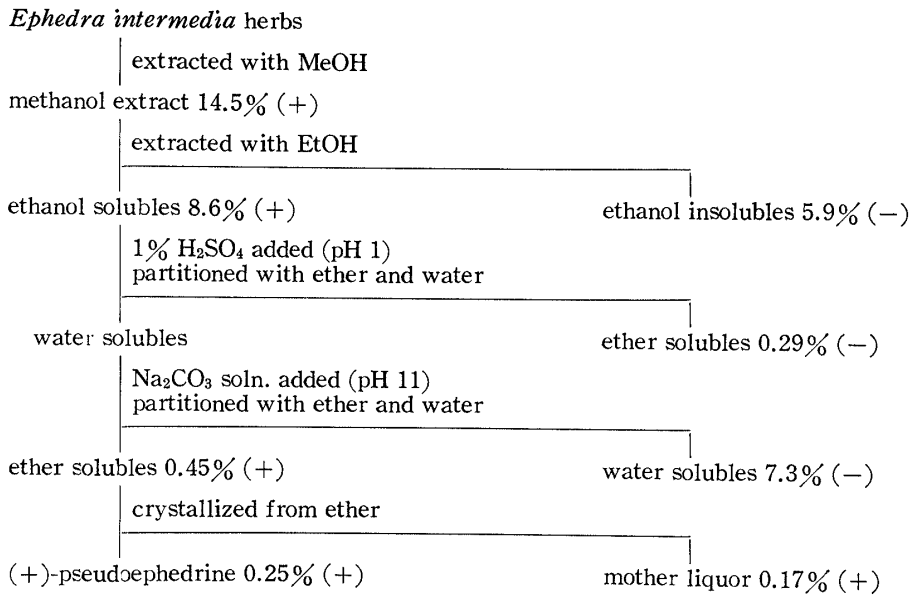

Chart 1. Flow Diagram of the Isolation of (+)-Pseudoephedrine from *Ephedra intermedia* Herbs

Yields (%) were calculated on the basis of the plant material and scores in parentheses represent the antiinflammatory activity of the fractions.

it deposited crystals. Crystallization yielded an active principle, which, on examination of its physico-chemical properties, was identified as (+)-pseudoephedrine. Another antiinflammatory principle, ephedroxane, has previously been obtained from the mother liquor.[6]

After elucidation of the antiinflammatory nature of (+)-pseudoephedrine, the antiinflammatory effects of the alkaloids of the ephedrine series were examined in different experimental models.

The activities of these alkaloids on the increase of vascular permeability induced by acetic acid in mice is shown in Table I. (+)-Pseudoephedrine exerted a dose-dependent activity which was the strongest tested, while (−)-ephedrine showed the weakest activity.

Table I. Effect of Ephedrine Analogs on Acetic Acid-induced Capillary Permeability in Mice

Substance	No. of mice	Inhibition (%) of capillary permeability Dose (mg/kg)				
		10	20	50	100	200
(−)-Ephedrine	5			4±13	26±10	18±12
(+)-ϕ-Ephedrine	5	2±18	24±12	42± 7*	50±14*	55±12**
(−)-N-Methylephedrine	5			28± 5*	39±13	40± 4**
(+)-N-Methyl-ϕ-ephedrine	5			12±11	17±17	47± 5**
Aminopyrine	5				45± 3**	

Significantly different from the control, $p<0.05$* or $p<0.01$**.

The effects of the ephedrine analogs on the mouse hind paw edema produced by carrageenin are presented in Fig. 1. In this assay, (−)-ephedrine exerted a dose-dependent activity. (+)-Pseudoephedrine showed similar activities at doses of 100 and 200 mg/kg. On the other hand, neither (−)-methylephedrine nor (+)-methylpseudoephedrine suppressed the foot swelling at the doses examined.

6) C. Konno, T. Taguchi, M. Tamada and H. Hikino, *Phytochem.*, **18**, 697 (1979).

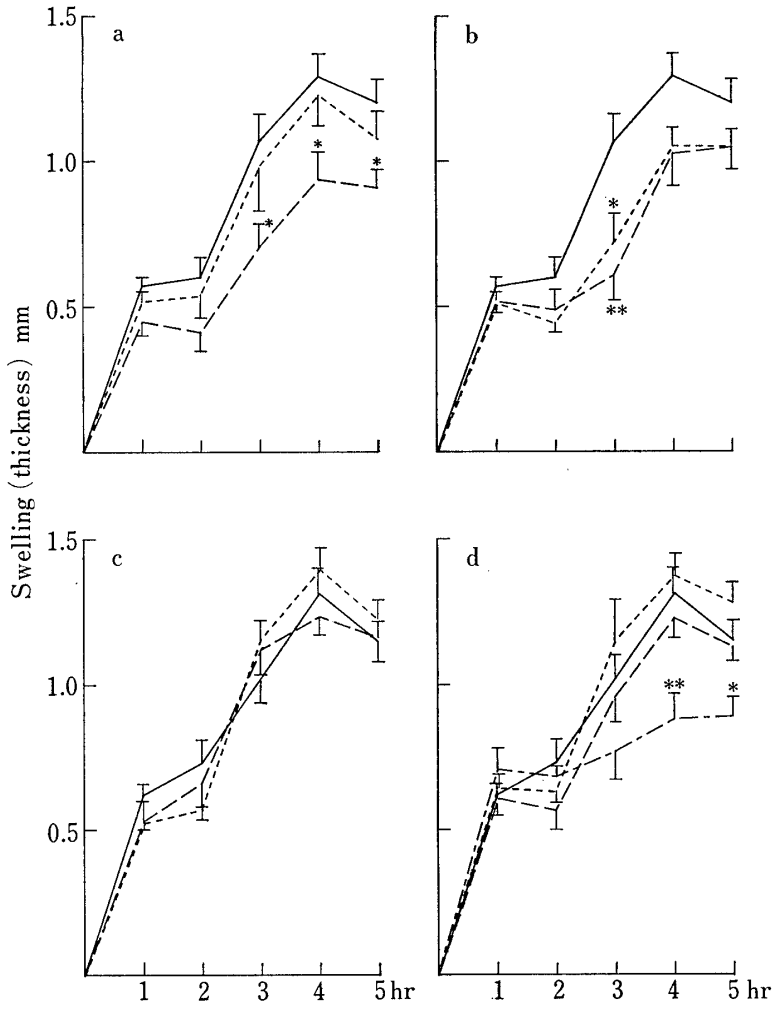

Fig. 1. Effect of Ephedrine Analogs on the Swelling of Mouse Hind Paw Edema induced by Carrageenin

a, (−)-ephedrine; b, (+)-pseudoephedrine; c, (−)-methylephedrine; d, (+)-methylpseudoephedrine.
———, control; ------, 100 mg/kg *p.o.*; – – –, 200 mg/kg *p.o.*; -·-·-, phenylbutazone 100 mg/kg *p.o.*
Significantly different from the control, $p<0.05$* or $p<0.01$**.

A protective effect on heat-induced erythrocyte lysis is known to be one of the biochemical indexes of antiinflammatory activity, and this is the only parameter which has been measured for ephedrine. In this system, Glenn *et al.*[7] found that (−)-ephedrine at a concentration of 41 μg/ml had no effect, while Aonuma *et al.*[8] observed that ephedrine hydrochloride showed dose-dependent activity in the concentration range of 25—100 μg/ml (22.5—43.9% inhibition). We investigated this discrepancy, and found that no ephedrine analog had a significant effect on heat-induced erythrocyte lysis at the doses employed (1—1000 μg/ml) (Table II).

The results for ephedrines in the fertile egg method are indicated in Table III. (+)-Pseudoephedrine reduced the amount of granulation tissue growing on the implanted disc at a high dose. On the other hand, (−)-ephedrine exhibited no particular activity at the doses examined in this assay.

Although ephedroxane was previously shown to be an antiinflammatory principle in *Ephedra* herbs, its content in the crude drug is small or zero, and its antiinflammatory activity

7) E.M. Glenn, B.J. Bowman and J.C. Koslowske, *Biochem. Pharmacol.*, Supplement, **17**, 27 (1968).
8) S. Aonuma, Y. Kohama, I.-J. Chen, S. Yashiki, S. Nakajin, and J. Sugatani, *Yakugaku Zasshi*, **95**, 151 (1975).

Table II. Effect of Ephedrine Analogs on Heat-induced Hemolysis of Rat Erythrocytes *in Vitro*

Substance	No. of rats	Inhibition (%) Concentration (μg/ml) 1	10	100	1000
(−)-Ephedrine	3	−18.7±4.0	4.4±1.0	−2.2±2.0	−5.5±2.2
(+)-ψ-Ephedrine	3	8.7±1.7	10.9±4.8	−1.7±5.8	3.0±3.4
(−)-N-Methylephedrine	3	−7.8±9.9	−15.2±5.2	−4.8±5.2	8.3±7.4
(+)-N-Methyl-ψ-ephedrine	3	−2.7±1.5	−1.1±1.5	1.6±1.3	−1.6±0.9
Indomethacin	3	8.9±3.0	42.0±1.0*	63.1±1.4*	

* Significantly different from the control, $p<0.01$.

Table III. Effect of Ephedrine Analogs on Granulation Tissue Formation in Chick Embryo

Substance	No. of eggs	Inhibition (%) of granulation tissue formation Dose (μg/disc) 12.5	25	50	100	250	500
(−)-Ephedrine	20		12	10	16	16	16
(+)-ψ-Ephedrine	20		10	16	10	28	48
Berberine chloride	20	59	65				

is rather weak, so it was concluded that ephedroxane could not account for the total antiinflammatory action of the crude drug.[6] Judging from its content in the crude drug and its physiological potency, it is now concluded that (+)-pseudoephedrine accounts for the antiinflammatory action of the crude drug.

The results obtained in this work provide a scientific basis for the traditional usage of the crude drug "maō" as an antiinflammatory.

Since the main antiinflammatory principle of the crude drug was elucidated to be (+)-pseudoephedrine, the contents of (+)-pseudoephedrine and (−)-ephedrine were estimated in the present preparation according to Yamasaki *et al.*[9] It was found that the preparation contained a fairly large amount of (+)-pseudoephedrine (0.64%) but relatively little (−)-ephedrine (0.11%). When the antiinflammatory activity of the methanol extract, and the activity and content of pseudoephedrine are taken into consideration, the former can be rationalized in terms of the latter in this preparation.

The finding that a preparation on the market contains quite a large amount of (+)-pseudoephedrine and a small amount of (−)-ephedrine raises the question of its therapeutic effectiveness. From the viewpoint of antiinflammatory activity, preparations containing large amounts of (+)-pseudoephedrine would be preferable, while on the other hand, (−)-ephedrine was reported to have a bronchodilative potency about 20 times greater than that of (+)-pseudoephedrine.[10] However, it was also said that the vasoconstrictor and bronchodilator actions of the two compounds are about the same, while the pressor, cardiac, mydriatic and central-stimulant actions of pseudoephedrine are weaker than those of ephedrine.[11] In fact, pseudoephedrine is clinically utilized more frequently than ephedrine

9) K. Yamasaki, K. Fujii, M. Sakamoto, K. Okada, M. Yoshida and O. Tanaka, *Chem. Pharm. Bull.*, **22**, 2898 (1974).
10) S. Sakai, M. Harada, I. Morishita and T. Kikuchi, *Yakugaku Zasshi*, **84**, 183 (1964).
11) "Remington's Pharmaceutical Sciences," 15th ed., ed. by A. Osol *et al.*, Mack Publishing Co., Easton, 1975, p. 820.

in the United States,[12] though this is not the case in Japan. This poses a problem about the quality of the crude drug "maō". Thus, provided that bronchodilator activity of pseudoephedrine is similar to that of ephedrine clinically, it may be concluded that crude drug preparations containing more pseudoephedrine is better in quality than those containing more ephedrine, because the former possesses more intense antiinflammatory activity. Since the clinical effectiveness and applicability of the crude drug "maō" will depend on the relative contents and activities of these two components, further studies on these aspects may be useful.

Experimental

^{1}H NMR spectrum was taken at 60 MHz. Chemical shifts (δ) are expressed in ppm downfield from TMS as internal reference and coupling constants (J) in Hz. Abbreviations: s=singlet, d=doublet, m=multiplet.

Assay for Antiinflammatory Activity——Effect on the increase of vascular permeability induced by acetic acid in mice (dd-strain) was determined according to Whittle.[13]

Measurement of activity on carrageenin-induced mouse hind paw edema was performed as follows. A group of 7 male mice of dd-strain (21—25 g) was used at each dose level. An alkaloid hydrochloride solution (10 ml/kg) was administered *p.o.* to mice, then after 30 min, 2% carrageenin in physiological saline solution (0.015 ml) was injected into the subplantar tissue of the right hind paw, and saline solution (0.015 ml) into the left hind paw. The difference in foot-pad thickness between the right and left feet was measured with a dial gauge calliper[14] every hr.

Effect on heat-induced hemolysis of the erythrocytes of rats (Wistar strain) was measured according to the method of Glenn *et al.*[7] as modified by Aonuma *et al.*[8]

The fertile egg method for assaying antiinflammatory activity utilizing the chorio-allantoic membrane of the chick embryo (White Leghorn strain) was carried out according to D'Arcy and Haward.[15] The total amount of granulation tissues at each dose level was calculated by subtraction of the total weight of the dried discs from that of the dried discs plus granulation tissues.

The results are expressed as mean±s.e.m.

Isolation of (+)-Pseudoephedrine from *Ephedra intermedia*——The crude drug "maō", the aerial part of *Ephedra intermedia* SCHRENKE *et* C.A. MEYER (6 kg), was extracted 5 times with cold MeOH (30 l) for 24 hr (each extraction). The solutions were combined and concentrated to give the MeOH extract (870 g), which was extracted with EtOH. The EtOH extract (424 g) was fractionated into acidic, neutral and basic portions in the usual manner. The basic portion (22.4 g) deposited crystals which, on crystallization from ether, gave (+)-pseudoephedrine as colorless prisms (12.3 g), mp 112—114° (uncorr.). $[\alpha]_D$ +48.7° (*c* 1.21, EtOH). *Anal.* Calcd for $C_{10}H_{15}NO$: C, 72.69; H, 9.15; N, 8.48. Found: C, 72.68; H, 9.08; N, 7.81. MS *m/e*: 166 (M^++1). IR ν_{max}^{KBr} cm^{-1}: 3315 (hydroxyl, amine); ^{1}H NMR ($CDCl_3$) δ: 0.87 (3H d, J 7.2, –CH–C<u>H</u>$_3$), 2.38 (3H s, N–C<u>H</u>$_3$), 2.61 (1H m, –CH–C<u>H</u>(NCH$_3$)–CH$_3$), 4.15 (1H d, J 7.8, C–C<u>H</u>(OH)–CH), 7.28 (5H s, aromatic <u>H</u>). Identification was based on in the usual criteria (mixed mp, TLC, IR and NMR spectra).

Acknowledgement We are grateful to Dr. M. Goto, Takeda Chemical Industries, Ltd., for performing some of the bioassays.

12) N.R. Farnsworth and A.S. Singel, "New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutical Activity," ed. by H. Wagner and P. Wolff, Springer-Verlag, Berlin Heidelberg New York, 1977, p. 3.
13) B.A. Whittle, *Brit. J. Pharmacol.*, **22**, 246 (1964).
14) S. Tsurufuji, K. Sugio and F. Takemasa, *Nature* (London), **280**, 480 (1979).
15) P.F. D'Arcy and E.M. Haward, *Brit. J. Pharmacol. Chemother.*, **29**, 378 (1967).