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EPHEDROXANE, ANTI-INFLAMMATORY PRINCIPLE OF *EPHEDRA* HERBS*

CHOHACHI KONNO, TAKASHI TAGUCHI, MITSURU TAMADA and HIROSHI HIKINO
Pharmaceutical Institute, Tohoku University, Aoba-yama, Sendai, Japan

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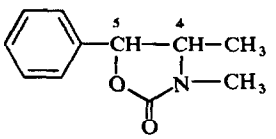
Key Word Index—*Ephedra intermedia*; *E. americana*; *E. andida*; *E. distachya*; *E. equisetina*; *E. gerardiana*; *E. minuta*; *E. sinica* ; *E. torreyana*; *E. tweediana*; Ephedraceae; alkaloid; ephedrine analog; ephedroxane; 3,4-dimethyl-5-phenyloxazolidone; anti-inflammatory activity.

The crude drug 'maō' is prepared from the aerial parts of certain species of *Ephedra* (Ephedraceae) and has been utilized for the perspiratory, antitussive, antipyretic, and anti-inflammatory purposes in Oriental medicine. Since the first isolation by Nagai [1] of (−)-ephedrine (**2**), which was later shown to have sympathomimetic and anti-allergic actions, its nor- and Me-analogs and the same set of analogs in the (+)-pseudoephedrine series [2] have been found in *Ephedra* herbs together with the other alkaloids, 2,3,4-trimethyl-5-phenyloxazolidine, 3,4-dimethyl-5-phenyloxazolidine [3], and benzylmethylamine [4].

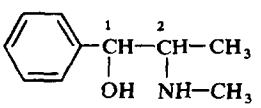
During the course of our investigation on the crude drug, which has been considered to possess an anti-inflammatory action as one of its therapeutic effects, we have found that the extract of an *Ephedra intermedia* herb exhibited this physiological activity. The survey for the active principles has led to the isolation of a new alkaloid designated as ephedroxane the structural determination of which is described in the present paper.

The MS of ephedroxane showed a M^+ peak at *m/e* 191, indicating that it has the composition $C_{11}H_{13}NO_2$. The IR spectrum exhibited a band at 1730 cm^{-1} due to carbonyl. In the PMR spectrum, there were a 3H doublet at 0.78 ppm for a C-Me, a 3H singlet at 2.91 ppm for an N-Me, a 1H multiplet at 4.05 ppm for a methine, a 1H doublet at 5.62 ppm for a methine and a 5H broad singlet at 7.40 ppm for aromatic hydrogens, the signals being in accord with those of ephedrine and pseudoephedrine except for slight displacement of the line positions. These PMR data demonstrated that ephedroxane was an ephedrine analog which was further shown to be an oxazolidone derivative (**1**) when the elemental composition and the IR carbonyl band were taken into consideration. In order to corroborate the conclusion chemically, (−)-ephedrine (**2**) and (+)-pseudoephedrine (**3**) were respectively converted according to the method reported by Sonoda *et al.* [5] into the two oxazolidone derivatives (**1** and **4**), the one from (−)-ephedrine being identical to the natural product. Although the oxazolidone (**1**) has already been synthesized by Hyne *et al.* [6], this is the first report of its natural occurrence.

The present work was originally initiated because the extract of the crude drug 'maō' showed anti-inflammatory activity, so that anti-inflammatory effect of ephedroxane (**1**) was examined by different assay procedures. In these



1 4*S*, 5*R*
4 4*S*, 5*S*



2 1*R*, 2*S*
3 1*S*, 2*S*

it was found that the compound did exhibit anti-inflammatory activity and the results will be reported elsewhere.

Our next interest was directed towards the content of the active principle ephedroxane in other *Ephedra* plants. The results of the assay performed on 15 samples from 10 species of *Ephedra* demonstrate that it is distributed widely in *Ephedra* plants which contain the ephedrine alkaloids (Table 1). There are, however, apparent exceptions. Thus, in certain *Ephedra* plants which contain the

Table 1. Ephedroxane content in *Ephedra* herbs

Original plant	Habitat	Ephedroxane content* (% dry wt)
E. americana	Kyoto, Japan (cult.)	—
E. andida	Kyoto, Japan (cult.)	—
E. distachya	Kyoto, Japan (cult.)	0.0012
E. distachya	Saitama, Japan (cult.)	0.00030
E. equisetina	Kyoto, Japan (cult.)	0.0011
E. equisetina	Saitama, Japan (cult.)	0.0021
E. gerardiana	Saitama, Japan (cult.)	0.00054
E. intermedia†	China (wild)	0.0017
E. intermedia	Saitama, Japan (cult.)	0.00064
E. intermedia	Saitama, Japan (cult.)	0.00036
E. minuta	Kyoto, Japan (cult.)	—
E. sinica	Kyoto, Japan (cult.)	—
E. sinica	Saitama, Japan (cult.)	0.0010
E. torreyana‡	Arizona, U.S.A. (wild)	—
E. tweediana‡	Kyoto, Japan (cult.)	—

* —: Not detected.
† Material from which ephedroxane was isolated.
‡ No ephedrine alkaloids detected.

* Part III in the series "Studies on the Constituents of *Ephedra*", For Part II see Tamada, M., Endo, K. and Hikino, H. (1978) *Planta med.* **34**, 291. This paper also constitutes Part 7 in the series on the validity of the Oriental medicines.

ephedrine alkaloids, no ephedroxane was detected. This may be explained by the fact that the content of ephedroxane in these plants was too small to be detected by the procedure employed or the plants lack an enzyme which converts ephedrine, the intermediate, into ephedroxane.

Since its content in the crude drug is small, if any, and furthermore its physiological activity is rather weak, ephedroxane may not represent the total anti-inflammatory action of the crude drug which, therefore, must be ascribed to some other principles.

EXPERIMENTAL

Isolation of ephedroxane from Ephedra *herb.* The crude drug Herba Ephedrae (6 kg), the dried aerial parts of *Ephedra intermedia* (Ephedraceae), were extracted overnight × 5 with MeOH (40 l. each time). The extract (830 g) was suspended in 1% H_2SO_4 and extracted with Et_2O (1 l. × 5). The aq. acidic soln was made alkaline (pH 11) with K_2CO_3 and extracted with Et_2O (1 l. × 5). Evapn of the Et_2O soln gave the alkaloid fraction (22.4 g) which was repeatedly chromatographed over Al_2O_3. Elution with $CHCl_3$–MeOH (20:1) furnished a crystalline fraction which on crystallization from EtOH afforded ephedroxane (1) as colorless needles (5 mg), mp 79–81° (uncorr.). MS *m/e*: 191 [M^+]. IR ν_{max}^{KBr} cm^{-1}: 1730 (carbonyl); PMR (100 MHz, CCl_4): δ 0.78 (3H, *d*, J = 7 Hz), 2.91 (3H, *s*), 4.09 (1H, *m*), 5.62 (1H, *d*, J = 8 Hz), 7.28 (5H, *br s*). Identity with the synthetic (4*S*,5*R*)-3,4-dimethyl-5-phenyloxazolidone (*vide post*) was confirmed by mp, mmp, MS, IR and PMR.

Preparation of (4S,5R)-3,4-*dimethyl-5-phenyloxazolidone from* (−)-*ephedrine.* To ephedrine (2) (300 mg) in DMF (20 ml), was added Et_3N (3 g) and Se powder (450 mg), and CO was bubbled in for 5 hr. Air was then bubbled through, the Se powder filtered off and the solvent evapd under red. pres. to yield the residue (325 mg). Chromatography over Si gel, elution with $CHCl_3$–MeOH (5:1) and crystallization from EtOH afforded (4*S*,5*R*)-3,4-dimethyl-5-phenyloxazolidone (1) as colorless needles (193 mg), mp 79–81° (uncorr.). MS *m/e*: 191 [M^+]. IR ν_{max}^{KBr} cm^{-1}: 1730 (carbonyl); PMR (60 MHz, CCl_4): δ 0.78 (3H, *d*, J = 7 Hz), 2.91 (3H, *s*), 4.09 (1H, *m*), 5.62 (1H, *d*, J = 8 Hz), 7.28 (5H, *br s*).

Preparation of (4S,5S)-3,4-*dimethyl-5-phenyloxazolidone from* (+)-*pseudoephedrine.* (+)-Pseudoephedrine (3) (385 mg) was treated as above and the product crystallized from EtOH to give (4*S*,5*S*)-3,4-dimethyl-5-phenyloxazolidone (4) as colorless prisms (214 mg), mp 50–51° (uncorr.). MS *m/e*: 191 [M^+]. IR ν_{max}^{KBr} cm^{-1}: 1737 (carbonyl); PMR (60 MHz, CCl_4): δ 1.33 (3H, *d*, J = 7 Hz), 2.84 (3H, *s*), 3.50 (1H, *m*), 4.88 (1H, *d*, J = 8 Hz), 7.30 (5H, *br s*).

Determination of ephedroxane content in Ephedra *herbs.* Each of the dried aerial parts of *Ephedra* plants (8 g) was extracted × 5 with MeOH (40 ml) for 8 hr. The MeOH extract (equivalent to 1 g of the dried plant material) was submitted to PLC on 0.5 mm thick layers for Si gel GF_{254} and development in $CHCl_3$. Extraction of the portion of the layer having R_f 0.13–0.46 (ephedroxane: R_f 0.28) with $CHCl_3$–MeOH (4:1) and evapn under red. pres. gave a residue which was dissolved in MeOH (0.1 ml). An aliquot (5 μl) of the soln was analysed by GLC on a 1 m × 2 mm column packed with 10% SE-30 at 150°. The content of ephedroxane was calculated from the areas of the peaks using a calibration curve previously prepared.

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