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HASUBANAN ALKALOIDS OF *STEPHANIA JAPONICA**

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Key Word Index—*Stephania japonica*, Menispermaceae, hasubanan alkaloids; 16-oxoprometaphanine, 16-oxohasubanonine; conversion of stephasunoline to metaphanine.

Abstract—Two new hasubanan alkaloids, 16-oxoprometaphanine and 16-oxohasubanonine, were obtained from *Stephania japonica* as minor components. A hasubanan alkaloid stephasunoline was converted into metaphanine *via* prometaphanine.

INTRODUCTION

In continuation of our studies on the non-quaternary alkaloids of *Stephania japonica* Miers, we have reported [1,2] the isolation and structural elucidation of four new hasubanan and one oxoaporphine alkaloids together with the eight known bases. In this paper, we wish to present the structures of two new congeners of the hasubanan series and conversion of the hasubanan alkaloid stephasunoline (**9**) [1] into metaphanine (**3**) [3]

RESULTS AND DISCUSSION

Isolation of a new hasubanan alkaloid from *Stephania japonica* collected in Kagoshima Prefecture, Japan, was reported earlier [1b] under the tentative name of base-P. Investigation on the structure of the base discussed below has shown that the new alkaloid was a hasubanan member and the base was named as 16-oxo-prometaphanine.

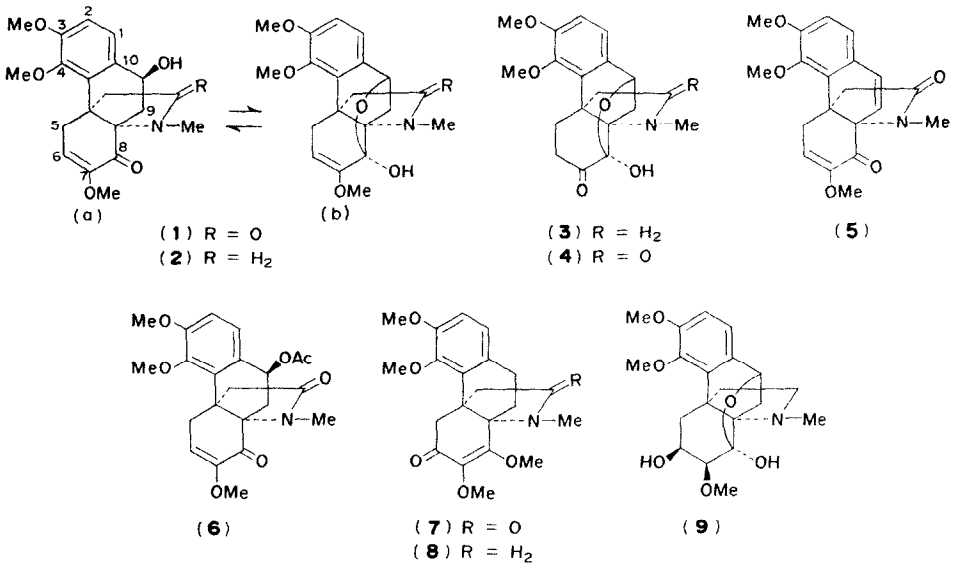
16-Oxoprometaphanine (**1**) $C_{20}H_{23}O_6N$ was crystallized from methanol as colorless prisms. The PMR spectra of the base in a variety of solvents, e.g. $CDCl_3$, $CDCl_3$–CCl_4 mixture, and pyridine-d_5, showed different signal patterns indicative of the presence of the equilibrium similar to the solvent-dependent equilibrium for prometaphanine (**2**) [4]. In acetone-d_6, the PMR spectrum of **1** showed three OMe groups at δ 3·96, 3·87, and 3·62, one *N*-Me group at δ 2·95, two vicinal aromatic protons at δ 7·35 (1H, doublet, *J* 8·5 Hz) and δ 7·01 (1H, doublet, *J* 8·5 Hz), and double doublet signals due to one olefinic proton at δ 6·01 (*J* 6·7 and 3·1 Hz). The IR spectrum of the base showed absorption bands of an OH group (3350–3310 cm^{-1}), a conjugated ketone and γ-lactam (1680 cm^{-1}) and an enolic double bond (1640 cm^{-1}). From these findings, **1** should be a γ-lactam alkaloid and the rational formula was given for the base.

On treatment with dil. HCl, the base (**1**) gave two compounds which were separated by preparative TLC (Al_2O_3). The strongly adsorbed compound was crystallized from ethanol as colorless prisms, mp 194°, $C_{20}H_{21}O_6N$, and corresponded with authentic 16-oxometaphanine (**4**), which was prepared by permanganate oxidation of metaphanine (**3**) [3]. The weakly adsorbed compound was crystallized from ethanol-hexane as colorless prisms, mp 157°, $C_{20}H_{21}O_5N$, and was identified as the styrene compound (**5**) [1].

These products indicate that 16-oxoprometaphanine (**1**) can assume both ketone form (**1a**) and ketal form (**1b**) to give the compounds **4** and **5**.

Treatment of 16-oxoprometaphanine (**1**) with acetic anhydride–pyridine gave 16-oxoprometaphanine acetate (**6**) as colorless prisms, mp 203°,

* Part 265 of the series "Studies on the Alkaloids of Menispermaceous Plants" Part 264, Watanabe, Y, Matsui, M, Iibuchi, M and Hiroe, S, (1975) *Phytochemistry* **14**, 2522



$C_{22}H_{25}O_7N$. Its IR spectrum showed absorption bands of acetate carbonyl group (1740 cm^{-1}), γ-lactam and conjugated ketone (1685 cm^{-1}), and an enolic double bond (1645 cm^{-1}). From the spectral data coupled with the fact [4] that a hemiketal hydroxyl group resists acetylation in the usual manner, it was assumed that the C(10)-hydroxyl group of the ketone form (**1a**) was acetylated but the C(8)-hemiketal hydroxyl group was not. This result suggested that equilibrium of the ketone (**1a**) and the ketal (**1b**) was shifted in the direction of **1a** in favor of the formation of **6**. Treatment of **6** with dilute HCl in the same manner as that described above gave **4** and **5**.

In view of the above facts, the most reasonable conclusion is that 16-oxoprometaphanine was the equilibrium mixture of the ketone (**1a**) and the ketal (**1b**) in solution similar to the equilibrium for prometaphanine (**2**) [4].

During the course of an investigation on the constituents of *S. japonica* native to the Amakusa Islands, Kumamoto Prefecture, Japan, a new minor hasubanan alkaloid 16-oxohasubanonine (**7**) was isolated. The base was crystallized from benzene–ether mixture as colorless prisms, mp 161°, $C_{21}H_{25}O_6N$. The MS fragmentation pattern of 16-oxohasubanonine (**7**) was similar to that of enone hasubanan alkaloids [5] showing the base ion peak (*m/e* 315) formed by loss of ring-D from the M^+ (*m/e* 387). The alkaloid was identical with an authentic sample of 16-oxohasubanonine which was derived from hasubanonine (**8**) [6] by permanganate oxidation. The natural occurrence of 16-oxohasubanonine (**7**), of which a synthesis was described earlier, [7] has been demonstrated for the first time.

Heating stephasunoline (**9**) in ethanol in the presence of sodium ethoxide gave a slightly yellow amorphous solid and this product was identical with prometaphanine (**2**), which was easily converted into metaphanine (**3**) [4]. This reaction by the basic reagent suggested that *trans*-elimination involving simultaneous removal of the C(6) β-axial OH group and the C(7) α-axial hydrogen of stephasunoline (**9**) resulted in the formation of prometaphanine (**2**). Therefore, the configuration of the C(6) hydroxyl and C(7) methoxyl groups of stephasunoline (**9**) discussed earlier on the PMR studies [1] was further supported by the above reaction. The reaction also took place on prolonged heating of stephasunoline (**9**) in chloroform with alumina.

EXPERIMENTAL

General procedures. Mp were uncorrected. PMR spectra were recorded at 60 MHz with TMS as an internal standard. Column chromatography was performed on neutral Al_2O_3 (activity II-III) and TLC was also carried out using this adsorbent. The alkaloids after TLC were detected by treatment with I_2 vapor and by spraying with Dragendorff's reagent.

16-Oxoprometaphanine (**1**). Colorless prisms, mp 195° (MeOH). $[\alpha]_D^{25}$ −52·0 (*c* = 0·5, $CHCl_3$). UV: λ_{max}^{MeOH} (ε) 230 (9500), 273 (8000) nm; IR: $\nu_{max}^{CHCl_3}$ 3550–3310 (OH), 1680 (conj. C=O and γ-lactam), 1640 (enolic C=C) cm^{-1}; PMR

$[(CD_3)_2CO]$· δ 2·95 (3H, *s*, NCH_3), 3·62, 3·87, 3·96 (each 3H, *s*, 3 × OMe), 4·40–4·78 (2H, diffused signal), 6·01 (1H, *dd*, *J* 6·7 and 3·1 Hz, C(6)-H), 7·01 (1H, *d*, *J* 8·5 Hz, aromatic H), 7·35 (1H, *d*, *J* 8·5 Hz, aromatic H), MS *m/e* 373 M^+, (46), 358 (M^+-15, 16), 275 (98), 258 (99), 257 (90), 242 (100) (Found C, 64·29, H, 6·47, N, 3·61 $C_{20}H_{23}O_6N$ requires C, 64·33, H, 6·21, N, 3·75%) TLC R_f 0·16 (Al_2O_3, $CHCl_3$), 0·62 (Si gel, MeOH–Me_2CO)

Treatment of 16-*oxoprometaphanine* (**1**) *with dil HCl* To a soln of **1** (86 mg) in MeOH (4 ml) was added 1 N HCl (0·5 ml) and the soln was heated for 5 min at 60° After removal of solvent, residue was dissolved in CH_2Cl_2 and the soln washed with H_2O and dried Removal of the solvent gave 78 mg of a colorless solid The solid was submitted to preparative TLC (Al_2O_3, $CHCl_3$) and separated into 2 compounds, R_f 0·22 and R_f 0·53 The compound R_f 0·22 was crystallized from EtOH to give 37 mg of colorless prisms, mp 194°, which was identical with an authentic sample of 16-oxometaphanine (**4**) prepared from metaphanine (**3**) as presented below The compound R_f 0·53 was crystallized from hexane–EtOH (4 1) mixture to give 29 mg of styrene compound (**5**) as colorless prisms, mp 157° This compound was identical with an authentic sample [1] of **5** in terms of mmp and a comparison of their IR ($CHCl_3$) and PMR ($CDCl_3$) spectra

16-*Oxometaphanine* (**4**) To a mixture of metaphanine (**3**) (103 mg), $MgSO_4$ (100 mg), Me_2CO (10 ml), and H_2O (20 ml) was added dropwise a solution of $KMnO_4$ (100 mg) in Me_2CO–H_2O (1 1) (30 ml) at 0° and the mixture was then stirred at room temp for 3 hr Excess reagent was decomposed with $NaHSO_3$ in 5% H_2SO_4 and the solvent was evaporated under red pres at room temp Residue was extracted with CH_2Cl_2 and the extract, after being washed successively with 2% HCl, 2% NaOH, and H_2O, was dried and removal of solvent gave 48 mg of a slightly yellow solid, which was chromatographed through a column (1 × 12 cm) of Si gel with $CHCl_3$ to give 36 mg of **4** as colorless prisms, mp 194° $[\alpha]_D^{28}$ −48·4° (*c* = 0·43, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ 3410 (OH), 1730 (six-membered C=O), 1685 (γ-lactam), NMR ($CDCl_3$) δ 3·04 (3H, *s*, NMe), 3·99 (6H, *s*, 2 × OCH_3), 5·06 (1H, *d*, *J* 6·5 Hz, C(10)-H), 5·12 (1H, *s*, C(8)-OH), 6·86 (2H, *s*, aromatic H) (Found C, 63·77; H, 6·03, N, 3·81 $C_{19}H_{21}O_6N$ requires C, 63·50; H, 5·89, N, 3·90%)

10-O-*Acetyl*-16-*oxoprometaphanine* (**6**) A mixture of 16-oxoprometaphanine (**1**) (64 mg), Ac_2O (1 ml), and pyridine (1 ml) was allowed to stand 18 hr at room temp Evaporation of solvent gave a slightly yellow oil, which was dissolved in CH_2Cl_2 and the soln was washed with H_2O and dried Removal of solvent gave a colorless solid, of which recrystallization from MeOH gave 55 mg of **6** as colorless prisms, mp 203°, IR $\nu_{max}^{CHCl_3}$ 1740 (AcO), 1685 (conj C=O, γ-lactam), 1645 (enolic C=C), NMR ($CDCl_3$) δ 2·11 (3H, *s*, OCOMe, 3·06 (3H, *s*, NCH_3), 3·63, 3·87, 3·95 (each 3H, *s*, 3 × OMe, *ca* 5·78 (2H, overlapped signal, C(6)-H, C(10)-H), 6·94 (2H, *s*, aromatic H) (Found C, 63·45, H, 6·11, N, 3·36 $C_{22}H_{25}O_7N$ requires C, 63·60, H, 6·07, N, 3·37%)

Treatment of 10-*O*-*acetyl*-16-*oxoprometaphanine* (**6**) *with dil HCl* To a soln of 10-*O*-acetyl-16-oxoprometaphanine (**6**) (35 mg) in MeOH (2 ml) was added 1 N HCl (0·3 ml) and the mixture was allowed to stand 18 hr at room temp After evaporation of solvent, residue was dissolved in CH_2Cl_2 and the soln was washed with H_2O and dried Removal of solvent gave 26 mg of a colorless solid The solid was treated with the same manner as that described above to provide 11 mg of **4** and 12 mg of **5**

Isolation of 16-*oxohasubanonine* (**7**) A MeOH extract (1·97 kg) was prepared from the dried chipped stems and rhizomes (10 kg) of *S. japonica* collected in March 1971 at Ushibuka-shi, the Amakusa Islands The extract was digested × 5 with 3% aq citric acid (total 36 l) and the acid soln shaken with $CHCl_3$ (total 49 l) to give the "weak base fraction" [1b] The $CHCl_3$ soln was shaken with 2% NaOH to remove phenolic bases, then the $CHCl_3$ layer was washed with H_2O and dried Removal of solvent gave 59 g of a non-phenolic extract The extract dissolved in C_6H_6 was chromatographed through a column (5 × 62 cm) of Al_2O_3 and elution with 500 ml of C_6H_6 gave 890 mg of hasubanonine (**8**), which was identified by comparison with an authentic sample [6] Successive elution with 1·3 l of the same solvent gave 37 mg of 16-oxohasubanonine (**7**)

16-*Oxohasubanonine* (**7**) Colorless prisms, mp 161° (C_6H_6–Et_2O) $[\alpha]_D^{23}$ −105·2° (*c* = 0·5, $CHCl_3$), MS *m/e* 387 (M^+), 372, 315 (100), 314, 283, 271, 257 (Found C, 65·35, H, 6·30; N, 3·73 Calc for $C_{21}H_{25}O_6N$ C, 65·10, H, 6·50, N, 3·62%) On admixture of the compound with an authentic sample [7] no mp depression was observed and the IR ($CHCl_3$) and PMR ($CDCl_3$) spectra of two compounds were superimposable

Conversion of stephasunoline (**9**) *into prometaphanine* (**2**) (i) To a soln of stephasunoline (**9**) (58 mg) in EtOH (5 ml) was added NaOEt (100 mg) and the mixture was refluxed for 20 min. The solvent was evaporated and H_2O (5 ml) was added to the residue. The mixture was extracted with $CHCl_3$ and the extract was washed with H_2O and dried Removal of solvent gave an oily residue, which was dissolved in $CHCl_3$ and chromatographed through a column (0·5 × 7 cm) of Al_2O_3 and eluted with the same solvent to give 36 mg of a slightly yellow amorphous solid Its IR spectrum ($CHCl_3$) was superimposable with that of an authentic sample [4] of prometaphanine (**2**) (ii) A mixture of **9** (54 mg) and Al_2O_3 (Brockmann, activity II–III) (2·1 g) in $CHCl_3$ (20 ml) was heated for 30 hr at 75–80° After cooling, the mixture was filtered and removal of solvent gave a slightly yellow oil, which was worked up in a similar fashion to that in (i) to give 32 mg of **2** as an amorphous solid

Metaphanine (**3**) *from prometaphanine* (**2**) A soln of prometaphanine (**2**) in Me_2CO was treated with the same procedure as that described in [4] to give metaphanine (**3**) as colorless prisms, which was identical with an authentic sample [3] of **3** (mmp, IR ($CHCl_3$) and PMR ($CDCl_3$))

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