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KANSHONE C, A SESQUITERPENOID OF *NARDOSTACHYS CHINENSIS* ROOTS*

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Key Word Index—*Nardostachys chinensis*; Valerianaceae; sesquiterpenoid; kanshone C.

Abstract—From the underground parts of *Nardostachys chinensis*, a novel aristolane-type sesquiterpenoid, kanshone C, was isolated along with the major constituent nardosinone. The structure of kanshone C was shown to be (1*S*, 10*S*)-8,9-dioxo-1,10-epoxyaristolane by spectral analysis.

INTRODUCTION

Nardostachys has been reported to possess aristolane-type sesquiterpenoids [1–5]. The roots and rhizomes of this genera have been used in Oriental medicine for sedative, aromatic, and stomachic purposes. In continuation of our earlier work on the roots of *Nardostachys chinensis* Batalin [6–8], we have isolated, in addition to the major constituent nardosinone (**2**), a novel highly oxidized aristolane-type sesquiterpenoid for which we propose the name kanshone C (**1**).

RESULTS AND DISCUSSION

Kanshone C was assigned the molecular formula $C_{15}H_{20}O_3$ from the molecular ion peak at *m/z* 248.1435 in its high resolution mass spectrum and the number of carbon signals in its ^{13}C NMR spectrum. The absence of olefinic carbon signal and the presence of two carbonyl carbon signals at δ 188.9 and 194.0 (each *s*) in the ^{13}C NMR spectrum required a tetracyclic skeletal system. The ^1H NMR signal at δ 3.65 (1H, *br s*) and ^{13}C NMR signals at δ64.7 (*s*) and 66.0 (*d*) suggested that the remaining oxygen atom was present as a trisubstituted oxirane ring. Further, the ^1H and ^{13}C NMR spectra indicated the presence of one secondary methyl group [δ 1.02 (3H, *d*, $J=6.8$ Hz) and δ 15.4 (*q*)] and three tertiary methyl groups [δ 1.21, 1.32, and 1.41 (each 3H, *s*), and δ 17.3, 19.5, and 30.5 (each *q*)]. Extensive ^1H NMR double resonance experiments, together with the two-dimensional ^1H–^{13}C shift correlation spectrum (C-H COSY) of kanshone C [9], gave the hydrogen sequence represented in part structure A.

In the ^{13}C NMR spectrum of kanshone C, two methine signals and one shielded quarternary signal appeared at δ 44.0 and 39.6 (each *d*), and δ 31.0 (*s*), respectively. The characteristically large J_{CH} values of 159.0 and 167.0 Hz for the first signals suggested the presence of a cyclopropane ring.

In order to settle the nature and environment of the two carbonyl groups present in the molecule, kanshone C was treated with sodium borohydride to afford the diol **3** (M^+ at *m/z* 252). The ^1H NMR spectrum of **3** displayed signals for three tertiary methyl groups at δ 1.08, 1.12, and 1.39 (each 3H, *s*), along with those due to the hydrogens of the part structure A. In addition to these signals, two newly generated carbinyl hydrogen signals were also discernible at δ 3.99 (1H, *d*, $J=5.6$ Hz) and 4.72 (1H, *dd*, $J=8.8$ and 5.6 Hz), which underwent downfield shifts to δ 5.41 (1H, *d*, $J=5.9$ Hz) and 5.65 (1H, *dd*, $J=9.9$ and 5.9 Hz), respectively in the diacetate **4** (M^+ at *m/z* 336). ^1H NMR double resonance experiments on the diol **3** revealed that the carbinyl hydrogen signal at δ 4.72 was coupled with the other carbinyl hydrogen signal at δ 3.99 ($J=5.6$ Hz) as well as with a triplet signal at δ 1.18 ($J=8.8$ Hz). Further, the latter signal (δ 1.18) was found to couple with a shielded doublet signal at δ 0.84 ($J=8.8$ Hz), both of which were assigned to be *cis* in a cyclopropane ring [10]. These results pointed to the presence of part structure B in the diol **3** which, in turn, established that kanshone C had an α-diketone functionality linked with a cyclopropane ring.

On the basis of the above findings and from the biogenetic consideration that the aristolane-type sesquiterpenoids have so far all been isolated from the same source [4, 5], the gross structure **1** (without stereochemistry) was proposed for kanshone C.

The relative stereochemistry of kanshone C was established by a study of the appropriate intramolecular nuclear Overhauser effects (NOE's). Thus, it was found that, when the H-14 and H-15 signals at δ 1.21 and 1.02, respectively, were separately irradiated, NOE's were detected for the H-6 signal at δ 2.02 (1H, *d*, $J=8.0$ Hz), demonstrating that H-6 was spatially close to these two methyl groups. Moreover, when the signal at δ 1.40 due to one of the C-11 methyl groups situated *trans* to the ring hydrogens on the cyclopropane was saturated, a significant NOE was observed for the H-1 signal at δ 3.65, revealing that they have the same orientations.

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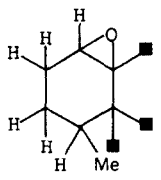

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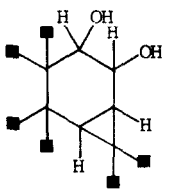

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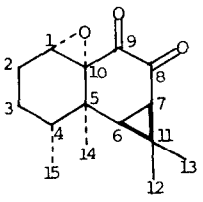

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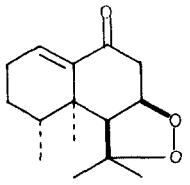

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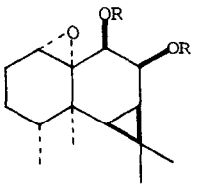

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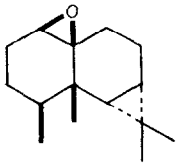
4 R=Ac



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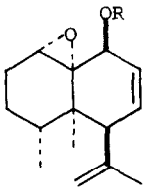

6 R=Ac
7 R=H
8 R=Bz

Regarding the relative configuration of the diol **3**, a spatial proximity was deduced between the C-5 methyl group and H-9 from the result of NOE studies which showed a positive NOE for the H-9 signal (δ 3.99) when the H-14 signal (δ 1.12) was irradiated. In addition, the coupling constant ($J = 5.6$ Hz) between the two carbinyl hydrogens (H-8 and H-9) inferred that they were *cis*.

Keeping in view of the *cis*-relationship of the diol moiety in formula **3**, the transformation of the diol **3** to the 8,9-dehydro derivative of 1,10-epoxyaristolane (**5**) [11] or its antipode was attempted in order to get the absolute configuration of kanshone C. Accordingly, the diol was treated with *N,N*-dimethylformamide dimethyl acetal followed by heating with acetic anhydride [12] to form the unexpected product **6** instead of the desired one. In the ^{1}H NMR spectrum of **6**, the signals due to the C-14 and C-15 hydrogens and an epoxide hydrogen appeared at δ 1.06 (3H, *s*), 0.76 (3H, *d*, $J = 6.7$ Hz), and 3.52 (1H, *d*, $J = 2.4$ Hz), respectively. Moreover, the ^{1}H NMR spectrum showed the absence of hydrogens on the cyclopropane ring and the presence of an acetoxyl methyl, a carbinyl hydrogen, two olefinic hydrogens, two hydrogens of an exocyclic methylene, a vinylic methyl, and one methine hydrogen flanked by two double bonds [δ 2.05 (3H, *s*), 5.45 (1H, *s*), 5.78 (2H, *br s*), 4.82 and 4.88 (each 1H, *s*), 1.68 (3H, *s*), and 3.03 (1H, *d*, $J = 2.5$ Hz). These data, along with the double resonance experiments carried out on the hydrogen signals, showed the genesis of the diene **6** by cleavage of the cyclopropane ring. The orientation of the acetoxyl group in the diene was shown to be opposite to that of the C-5 methyl group from the fact that a significant NOE was observed for the H-9 signal when the H-14 signal was irradiated. The monobenzoate (**8**) was prepared by alkaline hydrolysis of the diene (**6**) followed by benzoylation. The CD spectrum of the monobenzoate showed a positive Cotton effect at 231 nm ($\Delta\varepsilon$ +3.53) due to an interaction of the benzoyl chromophore with the Δ^7 double bond, demonstrating the *S* configuration at C-9. On the basis of above observations, we concluded the stereostructure of kanshone C to be (1*S*, 10*S*)-8,9-dioxo-1,10-epoxyaristolane (**1**).

Kanshone C (**1**) was screened for its possible antihepatotoxic activity by the cytotoxicity model system using primary cultured rat hepatocytes. It was found that kanshone C, at a dose of 0.1–1 mg/ml in the culture medium, showed a remarkable protective activity against D-galactosamine-induced liver damage in rat hepatocytes [13].

EXPERIMENTAL

Isolation of kanshone C. The dried roots of *Nardostachys chinensis* (1.5 kg) were extracted with CH_2Cl_2 (2 l × 3) for two days (each extraction) at room temp. The solvent was evapd off under red. pres. to afford a viscous brown oil (40 g) which was chromatographed over silica gel (600 g). The column was eluted with *n*-hexane and *n*-hexane–EtOAc mixtures of increasing polarity. Rechromatography of the *n*-hexane–EtOAc (9 : 1) eluate (2.0 g) over silica gel (200 g) using C_6H_6–EtOAc (19 : 1) as the solvent yielded, together with nardosinone (**2**) (0.1 g), kanshone C (**1**) (0.06 g) as yellow needles, mp 136–137°; $[\alpha]_D$ −13.4° ($CHCl_3$; *c* 0.40); EIMS (direct inlet) 70 eV, *m/z*: 248 $[M]^+$, 233, 205, 178, 164, 150, 135, 121, 107, 96, 95, 93, 83, 81, 67; UV λ_{max}^{MeOH} nm (log ε): 244 (3.4); IR ν_{max}^{KBr} cm^{-1}: 1715, 1687, 1450, 1375, 1235, 1110, 1080, 1050, 945, 800; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.02 (3H, *d*, $J = 6.8$ Hz, H-15), 1.21 (3H, *s*, H-14), 1.24 (1H, *dddd*, $J = 13.0$, 4.8, 2.9, and 2.5 Hz, H-3), 1.32 and 1.41 (each 3H, *s*, H-12 and H-13), 1.46 (1H, *dq*, $J = 4.6$ and 13.0 Hz, H-3), 1.67 (1H, *ddq*, $J = 13.0$, 2.9, and 6.8 Hz, H-4), 1.82 (1H, *dddd*, $J = 17.0$, 13.0, 4.8, and 0.8 Hz, H-2), 2.02 (1H, *d*, $J = 8.0$ Hz, H-6), 2.18 (1H, *ddt*, $J = 17.0$, 4.6, and 2.5 Hz, H-2), 2.32 (1H, *d*, $J = 8.0$ Hz, H-7), 3.65 (1H, *br s*, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 15.4 (*q*, C-15), 17.3 (*q*, C-12), 19.5 (*q*, C-13), 24.1 (*t*, C-3), 26.0 (*t*, C-2), 30.5 (*q*, C-14), 31.0 (*s*, C-11), 36.5 (*s*, C-5), 38.0 (*d*, C-4), 39.6 (*d*, C-7), 44.0 (*d*, C-6), 64.7 (*s*, C-10), 66.0 (*d*, C-1), 188.9 (*s*, C-8 or C-9), 194.0 (*s*, C-8 or C-9).

Reduction of kanshone C with $NaBH_4$. To a soln of kanshone C (**1**) (30 mg) in MeOH (5 ml), $NaBH_4$ (8 mg) was added and reaction mixture was stirred at room temp. for 1 hr. After the addition of H_2O, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried and chromatographed over silica gel (15 g). Elution with $CHCl_3$ yielded the diol **3** (20 mg) as colourless needles, mp 117–118°; $[\alpha]_D$ +12.3° ($CHCl_3$; *c* 0.78); EIMS (direct inlet) 70 eV, *m/z*: 252 $[M]^+$, 234, 222, 206, 205, 192, 178, 164, 150, 138, 123, 121, 109, 107, 95, 93, 91, 69, 42, 40; ^1H NMR (500 MHz, $CDCl_3$) δ: 0.84 (1H, *d*, *J* = 8.8 Hz, H-6), 0.88 (3H, *d*, *J* = 6.6 Hz, H-15), 1.05–1.15 (2H, *m*, H-3 and H-7), 1.08 and 1.12 (each 3H, *s*, H-14 and H-12), 1.37 (1H, *dq*, *J* = 3.7 and 13.0 Hz, H-3), 1.39 (3H, *s*, H-13), 1.70 (1H, *ddd*, *J* = 15.5, 2.8, and 13.0 Hz, H-2), 1.77 (1H, *ddq*, *J* = 13.0, 2.7, and 6.6 Hz, H-4), 2.05 (1H, *br dd*, *J* = 15.5 and 3.7 Hz, H-2), 3.49 (1H, *d*, *J* = 2.1 Hz. H-1), 3.99 (1H, *d*, *J* = 5.6 Hz, H-9), 4.72 (1H, *dd*, *J* = 8.8 and 5.6 Hz, H-8); ^1H NMR (500 MHz; Py-d_5) δ: 0.84 (1H, *d*, *J* = 8.8 Hz, H-6), 0.89 (3H, *d*, *J* = 6.9 Hz, H-15), 1.05 (1H, *br d*, *J* = 12.6 Hz, H-3), 1.10 (3H, *s*, H-12), 1.18 (1H, *t*, *J* = 8.8 Hz, H-7), 1.23 (3H, *s*, H-14), 1.55 (1H, *dq*, *J* = 3.6 and 12.6 Hz, H-3), 1.64 (3H, *s*, H-13), 1.70 (1H, *dt*, *J* = 12.6 and 3.6 Hz, H-2), 1.85 (1H, *ddq*, *J* = 12.6, 3.6 and 6.9 Hz, H-4), 2.00 (1H, *br d*, *J* = 12.3 Hz, H-2), 3.86 (1H, *d*, *J* = 3.1 Hz, H-1), 4.30 (1H, *d*, *J* = 5.5 Hz, H-9), 4.98 (1H, *dd*, *J* = 8.9 and 5.5 Hz, H-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 16.2 (*q*, C-15), 19.6 (*q*, C-12), 21.0 (*s*, C-11), 21.3 (*q*, C-13), 22.9 (*d*, C-6), 24.3 (*t*, C-3), 25.6 (*t*, C-2), 31.4 (*q*, C-14), 35.4 (*d*, C-7), 36.3 (*s*, C-5), 36.8 (*d*, C-4), 58.5 (*d*, C-1), 63.7 (*s*, C-10), 67.4 (*d*, C-8 or C-9), 68.2 (*d*, C-8 or C-9).

Acetylation of the diol. A soln of the diol (**3**) (5 mg) in Ac_2O (0.5 ml)-C_5H_5N (0.3 ml) was kept at room temp. overnight. Usual work-up of the reaction mixture afforded the diacetate **4** (5 mg) as colourless gum; EIMS (direct inlet) 70 eV, *m/z*: 336 $[M]^+$, 294, 281, 234, 219, 216, 201, 192, 191, 175, 160, 135, 137, 105, 85, 69, 54; IR ν_{max}^{NaCl} cm^{-1}: 1738, 1450, 1360, 1240, 1060, 940, 800; ^1H NMR (500 MHz, $CDCl_3$) δ: 0.88 (3H, *d*, *J* = 6.4 Hz, H-15), 0.92 (1H, *d*, *J* = 9.0 Hz, H-6), 1.05 (3H, *s*, H-14), 1.09 (1H, *br d*, *J* = 12.8 Hz, H-3), 1.19 (3H, *s*, H-12), 1.22(1H, *t*, *J* = 9.0 Hz. H-7), 1.33 (3H, *s*, H-13), 1.38 (1H, *dq*, *J* = 4.4 and 12.8 Hz, H-3), 1.67 (1H, *m*, H-2), 1.75 (1H, *ddq*, *J* = 12.8, 2.9, and 6.4 Hz, H-4), 1.97 (3H, *s*, COMe), 2.04 (1H, *m*, H-2), 2.05 (3H, *s*, COMe), 3.37 (1H, *d*, *J* = 2.3 Hz, H-1), 5.41 (1H, *d*, *J* = 5.9 Hz, H-9), 5.65 (1H, *dd*, *J* = 9.9 and 5.9 Hz, H-8).

Reaction of the diol with N,N-*dimethylformamide dimethyl acetal followed by* Ac_2O. The diol **3** (20 mg) was refluxed with *N,N*-dimethylformamide dimethyl acetal (3 ml) at 120° for 8 hr. Excess reagent was evapd under red. pres. and then the residue was treated with Ac_2O (2 ml) at 165° for 45 min. The Ac_2O was removed under red. pres. and the gummy residue chromatographed over a silica gel column (20 g). Elution with *n*-hexane–EtOAc (19 : 1) afforded the diene 6 as colourless needles (6 mg); mp 75°; EIMS (direct inlet) 70 eV, *m/z*: 276 $[M]^+$, 234, 216, 201, 173, 160, 152, 150, 137, 131, 109, 105, 98, 95, 82, 79, 77, 69, 55; UV λ_{max}^{MeOH} nm (log ε): 209 (3.7); IR ν_{max}^{NaCl} cm^{-1}: 1735, 1448, 1360, 1220, 1020, and 880; ^1H NMR (500 MHz, $CDCl_3$) δ: 0.76 (3H, *d*, *J* = 6.7 Hz, H-15), 1.06 (3H, *s*, H-14), 1.68 (3H, *s*, H-11), 2.05 (3H, *s*, COMe), 2.94 (1H, *d*, *J* = 2.9 Hz, H-6), 3.52 (1H, *d*, *J* = 2.4 Hz, H-1), 4.82 and 4.88 (each 1H, *s*, H-12), 5.45 (1H, *s*, H-9), 5.78 (2H, *br s*, H-7 and H-8); ^{13}C NMR (25 MHz, $CDCl_3$) δ: 15.4 (*q*, C-15), 18.7 (*q*, C-13), 19.1 (*q*, C-14), 21.1 (*q*, COMe), 23.8 (*t*, C-3), 26.1 (*t*, C-2), 34.3 (*d*, C-4), 38.9 (*s*, C-5), 55.8 (*d*, C-6), 60.3 (*d*, C-1), 63.1 (*s*, C-10), 72.5 (*d*, C-9), 115.3 (*t*, C-12), 124.0 (*d*, C-7 or C-8), 132.0 (*d*, C-7 or C-8), 144.5 (*s*, C-11), 169.8 (*s*, COMe).

Alkaline hydrolysis of the diene. To a soln of diene **6** (2.5 mg) in MeOH (2 ml), 0.5 M K_2CO_3 (0.3 ml) was added and the reaction mixture was stirred at room temp. overnight. The reaction mixture was neutralized with 0.5 M H_2SO_4 and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and evapd to afford the alcohol **7** (2 mg) as colourless gum; EIMS (direct inlet) 70 eV, *m/z*: 234 $[M]^+$, 218, 216, 201, 188, 173, 160, 154, 145, 135, 121, 119, 109, 107, 105, 98, 95, 93, 91, 82, 80, 77, 69, 67, 54; ^1H NMR (100 MHz, $CDCl_3$) δ: 0.78 (3H, *d*, *J* = 6.5 Hz, H-15), 1.04 and 1.62 (each 3H, *s*, H-14 and H-12), 2.0 (1H, *m*, H-2), 2.95 (1H, *d*, *J* = 5.6 Hz, H-6), 3.74 (1H, *d*, *J* = 2.2 Hz, H-1), 4.18 (1H, *br s*, H-9), 4.82 (2H, *br d*, *J* = 4.2 Hz, H-12), 5.70 (1H, *ddd*, *J* = 10.0, 5.6, and 1.2 Hz, H-7), 5.80 (1H, *dd*, *J* = 10.0 and 3.2 Hz, H-8).

Benzoylation of the alcohol. A soln of the alcohol **7** (2.0 mg) in C_6H_5COCl (0.1 ml) and pyridine (0.2 ml) was kept at room temp. overnight. The excess C_6H_5COCl was destroyed by the addition of H_2O and extracted with EtOAc. The EtOAc layer was washed with H_2O and dried to afford gummy residue, which was chromatographed over silica gel (5 g). On elution with C_6H_6 the monobenzoate **8** was obtained (1.5 mg) as colourless gum; CD (MeOH; *c* 0.087): $\Delta\varepsilon_{207}$ −13.0, $\Delta\varepsilon_{231}$ +3.53; EIMS (direct inlet) 70 eV, *m/z*: 233 $[M-C_6H_5CO]^+$, 216, 201, 183, 159, 152, 105, 91, 85; UV λ_{max}^{MeOH} nm (log ε): 207 (4.03), 230 (4.02); IR ν_{max}^{NaCl} cm^{-1}: 1720, 1470, 1450, 1260, 1100, 1030, 800; ^1H NMR (500 MHz, $CDCl_3$) δ: 0.79 (3H, *d*, *J* = 6.3 Hz, H-15), 1.10 and 1.80 (each 3H, *s*, H-14 and 13), 2.05 (1H, *m*, H-2), 3.10 (1H, *d*, *J* = 6.0 Hz, H-6), 3.61 (1H, *d*, *J* = 2.9 Hz, H-1), 4.86 and 4.92 (each 1H, *s*, H-12), 5.72 (1H, *d*, *J* = 4.3 Hz, H-9), 5.87 (1H, *ddd*, *J* = 10.0, 6.0, and 1.1 Hz, H-7), 5.93 (1H, *dd*, *J* = 10.0 and 4.3 Hz, H-8), 7.45 (2H, *dd*, *J* = 7.0 and 1.0 Hz, C_6H_5CO), 7.56 (1H, *t*, *J* = 7.0 Hz, C_6H_5CO), 7.98 (2H, *d*, *J* = 7.0 Hz, C_6H_5CO).

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REFERENCES

1. Büchi, G., Greuter, F. and Tokoroyama, T. (1962) *Tetrahedron Letters* 827.
2. Pernelle, P. and Ourisson, G. (1963) *Bull. Soc. Chim. Fr.* 518.
3. Sastry, S. D., Maheswari, M. L., Chakravarti, K. K. and Bhattacharya, S. C. (1967) *Tetrahedron* **23**, 1997.
4. Rücker, G. (1968) *Liebigs Ann. Chem.* **717**, 221.
5. Rücker, G. and Kretzschmar, U. (1971) *Liebigs Ann. Chem.* **748**, 214.
6. Hikino, H., Hikino, Y., Koakutsu, S. and Takemoto, T. (1972) *Phytochemistry* **11**, 2097.
7. Bagchi, A., Oshima, Y. and Hikino, H. (1988) *Planta Med.* **54**, 87.
8. Bagchi, A., Oshima, Y. and Hikino, H. (1988) *Phytochemistry* **27**, 1199.
9. Maudsley, A. A., Muller, L. and Ernst, R. R. (1977) *J. Org. Reson.* **28**, 463.
10. Jackmann, L. M. (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* 2nd Edn, p. 286. Pergamon Press, Oxford.
11. Do, M. N. and Erickson, K. L. (1983) *J. Org. Chem.* **48**, 4410.
12. Eastwood, F. W., Harigton, K. J., Josan, J. S. and Pura, J. L. (1970) *Tetrahedron Letters* 5223.
13. Kiso, Y., Tohkin, M. and Hikino, H. (1983) *J. Nat. Prod.* **46**, 841.