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A DITERPENOID PHENALENONE FROM *SALVIA MILTIORRHIZA*

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Key Word Index—*Salvia miltiorrhiza*; Labiatae; diterpenoids; phenalenones; ^{13}C NMR.

Abstract—A phenalenone isolated from *Salvia miltiorrhiza* was identified as 9-isopropyl-2,2,5-trimethyl-8H-phenaleno[1,9bc]furan-8-one, a compound which had been artificially prepared by rearrangement of taxoquinone and other royleanones.

INTRODUCTION

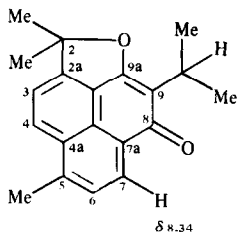
Tan-shen is the dried roots of a medicinal plant, *Salvia miltiorrhiza* L., that is commonly used in China. It is red externally, and because of its colour it is used in folk medicine for the treatment of all blood difficulties, hemorrhages, menstrual disorders and miscarriages. A number of diterpenoid pigments possessing phenanthraquinone and naphthaquinone chromophores have been isolated from Tan-shen, as exemplified by tanshinone-I [1] and II [2]. In the course of our study on the minor components of Tan-shen, we isolated several diterpenoids [3], together with a bright yellow pigment. The physical properties of this yellow substance were found to be quite different from those of the naphthaquinones and phenanthraquinones present in this plant. We have now elucidated the structure of the yellow pigment, salvilenone (**1**), by using modern spectroscopic techniques, and shown it to have a unique phenalenone framework. The same compound has been prepared by Eugster *et al.* by rearrangement of taxoquinone (**4**) and other royleanones [4].

RESULTS AND DISCUSSION

Salvilenone (**1**), mp 141.2°, $C_{20}H_{20}O_2$, was found to be a very stable crystalline material; no decomposition occurred during 14 years storage at room temperature. Its UV absorption maxima differed from those of simple benzo-, naphtha-, or phenanthraquinones. An intense IR band at 1610 cm^{-1} was attributed to a carbonyl group, the π-electron of which was highly delocalized by conjugation. The other oxygen atom was deduced to be involved in an ether linkage, because no hydroxyl band was observed in the IR spectrum. The major difficulty in the structure elucidation of salvilenone was in the analysis of the ^1H NMR spectrum which showed only a few groups of signals ascribable to the substructures **a**–**d**, and, because they were located separately, their correlation using a ^1H–^1H homonuclear spin decoupling method was impossible. The structure (**1**) of salvilenone was obtained by analysing the long-range couplings between the protons and carbons, which were suggested by a heteronuclear (^1H–^{13}C) low-power selective proton decoupling (LSPD) technique [5]. Thus, the substructures **a** and **b** were extended to the partial structure **A** by a decoupling study in which the olefinic methyl signal at δ2.78 was weakly irradiated. This resulted in the following changes; (1) a multiplet at δ140.5 (C_Y) was changed into a double doublet (J = 7.5, 4.0 Hz), (2) a double quartet at δ128.8 (C_Z, J = 161.0, 5.5 Hz) was changed into a doublet (J = 161.0 Hz), and (3) a multiplet at δ130.1 (C_X) was changed into a triplet (J = 7.5 Hz).

Similarly, the partial structure **B** was deduced by irradiations of the methine (δ3.43) and the *gem*-dimethyl (δ1.75) signals. Throughout the decoupling studies, three quarternary carbon signals at δ123.2 (C-9c; *dd*, J = 7.5 Hz), 125.7 (C-9b; *d*, J = 7 Hz), and 127.8 (C-7a; *d*, J = 7.0 Hz) were unaffected.

When the ^1H NMR spectrum of salvilenone (**1**) was taken in C_6D_6, downfield shifts (compared to those in $CDCl_3$) were observed only for the signals due to the



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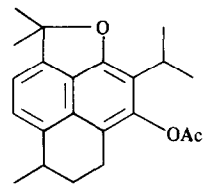

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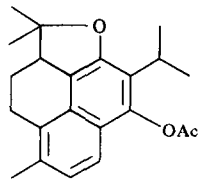

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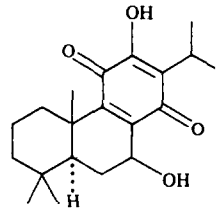

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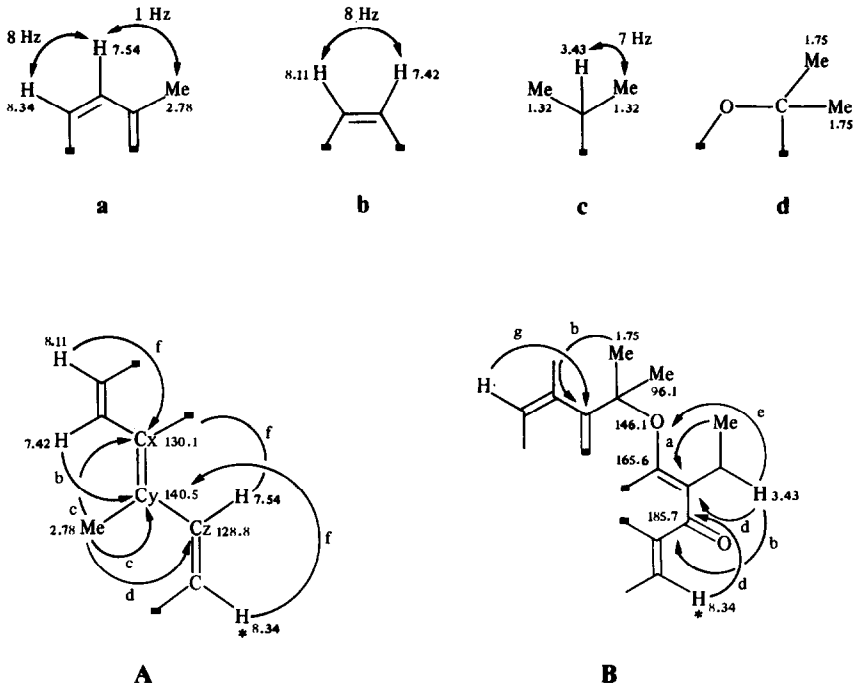

J^{CH} (Hz): a; 2.5, b; 4.0, c; 5.0, d; 5.5, e; 6.0, f; 7.5, g; 8.0

isopropyl group and the aromatic proton at δ8.34, indicating that these fragments were situated on the 'front side' of the carbonyl group [6], and that the protons denoted by asterisks (*) in **A** and **B** were identical. On the basis of these findings, the structure **1** was deduced for salvilenone. All the ^1H–^{13}C coupling patterns were fully consistent with the structure **1** (see Experimental).

On hydrogenation, salvilenone (**1**) gave unstable products, which were smoothly oxidized by air back to **1**. The hydrogenation products could be obtained as tetrahydroacetates by hydrogenation on palladium–charcoal in methanol, followed by evaporation of the solvent *in vacuo* and immediate acetylation under an argon atmosphere. On the basis of the spectral analyses, the structures **2** and **3** were assigned to the tetrahydroacetates (**2**:**3**::7:1). Both acetates afforded **1** quantitatively on saponification followed by air oxidation of the resulting hydrolysed products.

In 1975, Eugster *et al.* obtained a yellow product by dehydration of taxoquinone (**4**) and other royleanones with 80% sulphuric acid at 0°. By means of X-ray analysis, they determined the structure of the product as **1**, the physical properties of which are the same as those of salvilenone. Since our extraction and isolation procedures were quite mild (neutral pH and room temperature) salvilenone (**1**) is considered to be a natural product even though possible precursors such as taxoquinone (**4**) are present in *S. miltiorrhiza*.

EXPERIMENTAL

Isolation of salvilenone (**1**). Dried roots of *Salvia miltiorrhiza* were supplied by Sankyo Company, Ltd., Hiromachi, Shinagawa, Tokyo. An Et_2O extract (135 g) of the roots (14 kg) was fractionated by repeated chromatography on Al_2O_3 (Merck, Art. 1097) using C_6H_6 as eluting solvent. A yellow-coloured fraction eluted after the tanshinone-II fraction, was collected, and further fractionated by prep. TLC (Merck, Kieselgel 60 F_{254}, $CHCl_3$) to give a bright yellow solid which crystallized from hexane to give salvilenone (**1**, 35 mg), mp 141.2°. IR, UV and ^1H NMR spectra essentially identical with those reported in ref. [4]. High-resolution EIMS (direct inlet) *m/z*: $[M]^+$ 294.1406 (calc. 292.1462 for $C_{20}H_{20}O_2$); ^{13}C NMR (22.5 MHz; $CDCl_3$): δ185.7 (*dd*, C-8, $J_{8C,7H}$ = 5.5 Hz, $J_{8C,9CH}$ = 4.0 Hz), 165.6 (*d*, C-9a, $J_{9aC,9CH}$ = 6.0 Hz), 146.1 (*m*, C-2a, $J_{2aC,2Me}$ = 4.0 Hz, $J_{2aC,4H}$ = 8.0 Hz), 140.5 (*m*, C-5, $J_{5C,4H}$ = 4.0 Hz, $J_{5C,5Me}$ = 5.0 Hz, $J_{5C,7H}$ = 7.5 Hz), 130.1 (*m*, C-4a, $J_{4aC,3H}$ = 7.5 Hz, $J_{4aC,5Me}$ = 5.0 Hz, $J_{4aC,6H}$ = 7.5 Hz), 129.5 (*d*, C-7, $J_{7C,7H}$ = 165.0 Hz), 128.8 (*dq*, C-6, $J_{6C,6H}$ = 161.0 Hz, $J_{6C,5Me}$ = 5.5 Hz), 127.8 (*d*, C-4, $J_{4C,4H}$ = 161.0 Hz), 127.8 (*d*, C-7a, $J_{7aC,6H}$ = 7.0 Hz), 125.7 (C-9b, $J_{9bC,3H}$ = 7.0 Hz), 123.2 (*dd*, C-9c, $J_{9cC,4H}$ = 7.5 Hz, $J_{9cC,7H}$ = 7.5 Hz), 119.7 (*m*, C-9, $J_{9C,9CH}$ = 5.5 Hz, $J_{9C,9CMe}$ = 2.5 Hz), 118.4 (*d*, C-3, $J_{3C,3H}$ = 163.0 Hz), 96.1 (*m*, C-2, $J_{2C,2Me}$ = 4.0 Hz), 26.9 (*q*, iso-propyl Me_2), 24.3 (*d*, iso-propyl CH), 21.0 (*q*, 2-Me_2), 19.1 (*q*, 5-Me).

Reductive acetylation of salvilenone (**1**). Salvilenone (**1**, 5 mg) was hydrogenated (Pd) in MeOH (0.5 ml) for 2 hr. The solvent was removed, and the residue acetylated (Ac_2O–pyridine) under an argon atmosphere to give a yellow solid (5.8 mg) after the usual work-up. Separation by HPLC (YMC Pack A-012, hexane–EtOAc, 97:3) afforded oily **2** (4 mg) and **3** (0.6 mg) together with **1** (0.6 mg).

Compound **2**. UV λ_{max}^{MeOH} nm (log ε): 212 (4.49), 238 (4.62), 302 sh (3.86), 314 (3.90), 330 (3.95); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (AcO), 1628, 1597 (aromatic ring); ^1H NMR (90 MHz, $CDCl_3$, TMS as int. standard): δ1.35 (9H, *d*, *J* = 7.2 Hz, 5-Me and iso-propyl

Mes), 1.66 (6H, *s*, 2-Me_2), 1.6–2.3 (2H, *m*, H-6), 2.36 (3H, *s*, Ac), 2.6–2.9 (2H, *m*, H-7), 3.03 (2H, *m*, H-5 and iso-propyl CH), 6.95 (1H, *d*, J = 7.2 Hz, H-3), 7.17 (1H, *dd*, J = 7.2, 1.2 Hz, H-4); ^{1}H NMR (90 MHz, C_6D_6, TMS as int. standard): δ1.31 (3H, *d*, J = 7.2 Hz, 5-Me), 1.56 (6H, *s*, 2-Me_2), 1.74 (6H, *d*, J = 7.2 Hz, $CHMe_2$), 1.6–2.1 (2H, *m*, H-6), 2.05 (3H, *s*, Ac), 2.6–3.2 (3H, *m*, H-5 and H-7), 3.47 (1H, *hept*, J = 7.2 Hz, $CHMe_2$), 6.84 (1H, *d*, J = 7.2 Hz, H-3), 7.21 (1H, *dd*, J = 7.2, 1.2 Hz, H-4). High-resolution EIMS (direct inlet) m/z: $[M]^+$ 338.1879 (calc. 338.1882 for $C_{22}H_{26}O_3$).

Compound **3**. UV λ_{max}^{MeOH} nm (log ε): 218 (4.47), 243 (4.71), 278 sh (3.61), 290 (3.65), 304 (3.61), 325 sh (3.36), 340 (3.44); ^{1}H NMR (90 MHz, $CDCl_3$): δ1.18 (3H, *s*, 2-Me), 1.27 (3H, *d*, J = 7.2 Hz, *iso*-propyl Me), 1.33 (3H, *d*, J = 7.2 Hz, isopropyl Me), 2.35 (3H, *s*, 5-Me), 2.44 (3H, *s*, Ac), 2.4–4.6 (4H, *m*, H-2a, H-4, *iso*-propyl CH), 7.04 (1H, *d*, J = 9 Hz, H-6 or H-7), 7.27 (1H, *d*, J = 9 Hz, H-7 or H-6). High-resolution EIMS (direct inlet) m/z: $[M]^+$ 338.1879 (calc. 338.1882 for $C_{22}H_{26}O_3$).

Hydrolysis of **2** *and* **3**, *and subsequent air oxidation to* **1**. A soln of **2** (or **3**) (50 μg) in MeOH–H_2O (1:1) (0.2 ml) was treated with two microdrops of 5 M NaOH, and the mixture was allowed to stand overnight. After acidification, the product was taken up in Et_2O. The Et_2O was evaporated and the residue was dissolved in Me_2CO. Analysis by GC (OV-1, FID, He 15 ml/min, 1 m × 3 mm packed with OV-1) revealed the quantitative formation of salvilenone (**1**).

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24-METHYLENE CYCLOARTANYL *p*-HYDROXYCINNAMATE FROM THE ORCHID *CIRRHOPETALUM ELATUM*

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Key Word Index—*Cirrhopetalum elatum*, Orchidaceae; 24-methylenecycloartanyl *p*-hydroxycinnamate; triterpene.

Abstract—From the orchid *Cirrhopetalum elatum* was isolated a new triterpene of the cycloartane series, which was shown to be 24-methylenecycloartanyl *p*-hydroxycinnamate from spectral and chemical evidence.

INTRODUCTION

Systematic chemical investigations of a series of Himalayan orchids in our laboratory earlier led to the isolation of several 9,10-dihydrophenanthropyrans and pyrones, 9,10-dihydrophenanthrenes, phenanthrenes, bibenzyl derivatives and steroids [1, 2]. We report in this paper the isolation of a new triterpene from yet another Himalayan orchid *Cirrhopetalum elatum*. The triterpene was shown to be 24-methylenecycloartanyl *p*-hydroxycinnamate (**1a**) from the following spectral and chemical evidence.

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RESULTS AND DISCUSSION

Elemental analysis of the triterpene, mp 255°, $[\alpha]_D$ +28.3° ($CHCl_3$), corresponded to a molecular formula $C_{40}H_{58}O_3$ which was confirmed by its chemical ionization mass spectrum showing a peak at m/z 587 $[M+1]^+$. However, it did not show the molecular ion peak in its electron-impact mass spectrum which, instead, exhibited a peak at m/z 422 $[M - C_9H_8O_3]$ at the highest mass region corresponding to the loss of *p*-hydroxycinnamic acid.

The presence of the *p*-hydroxycinnamate ester moiety in the compound was indicated by its characteristic UV [λ_{max} 213, 228 and 314 nm (log ε3.99, 4.02 and 4.33); large alkali-induced bathochromic shifts, $\lambda_{max}^{0.1\,N\,NaOH}$ 242 and 367 nm (log ε3.82 and 4.45)], IR [ν_{max} 3190 (OH), 1675 (conjugated C=O), 985 (*trans*-CH=CH–) and 830 (1,4-