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STRUCTURES OF TRIPTOFORDININE A-1 AND A-2 DETERMINED BY TWO-DIMENSIONAL NMR
 SPECTROSCOPY. HIGHLY ESTERIFIED SESQUITERPENE ALKALOIDS FROM
TRIPTERYGIUM WILFORDII HOOK FIL. VAR. REGELII MAKINO

Yoshihisa Takaishi,^{*,a} Kunie Ujita,^a Hiroyasu Noguchi,^a Kimiko Nakano,^a
 Toshiaki Tomimatsu,^a Shigetoshi Kadota,^b Koji Tsubono,^b and Tohru Kikuchi^{*,b}

Faculty of Pharmaceutical Sciences, University of Tokushima,^a Shomachi, Tokushima
 770, Japan and Research Institute for Wakan-Yaku (Oriental Medicines), Toyama
 Medical and Pharmaceutical University,^b 2630 Sugitani, Toyama 930-01, Japan

The structures of triptofordinine A-1 and A-2, highly esterified sesquiterpene alkaloids isolated from *Tripterygium wilfordii* Hook fil. var. *regelii* Makino, were established by the application of two-dimensional NMR.

KEYWORDS— sesquiterpene; alkaloid; triptofordinine; two-dimensional NMR; celastraceae; tripterygium wilfordii

The sesquiterpene dihydroagarofuran (1) occurs in nature as euonymiol (2) in various oxygenated forms, bearing as many as nine hydroxy groups.¹⁾ These polyols occur mostly in members of the *Celastraceae* plants as polyesters, in which the constituent acids are aliphatic (acetic, 2-methylbutyric, hydroxyisobutyric, etc.) and/or aromatic (benzoic, cinnamic, furoic, nicotinic, etc.).²⁾ In the structural elucidation of these sesquiterpenes, the difficulty of determining the linking sites of respective ester groupings when more than three kinds of acids are involved as esters in the molecule necessitates the use of the X-ray crystallographic method^{3,4)} or selective hydrolyses.⁵⁾ In fact, determination of the estersites in such compounds has been unsuccessful in many cases.^{3,6)} This problem can be solved conveniently by using 2-D NMR spectroscopy including ¹H-¹³C long-range COSY.⁷⁾ Here we describe the structural elucidation of two new sesquiterpene alkaloids, triptofordinine A-1 (3) and A-2 (4), from *Tripterygium wilfordii* Hook fil. var. *regelii* Makino,⁸⁾ which is used in China as a drug for the treatment of cancer and as an insecticide.⁹⁾

The methanol extract from the leaves of *T. wilfordii* var. *regelii* was separated by a combination of silica gel and Sephadex LH-20 column chromatography and HPLC to give five new sesquiterpene alkaloids: triptofordinine A-1, A-2, A-3, B, and C.

In its MS, triptofordinine A-1 (**3**), $C_{41}H_{43}NO_{12}$, mp 193-194°C, $[\alpha]_D -81.4^\circ$ (MeOH), UV λ_{max} nm (ϵ): 220(36000), 275(17700), 282(17500), had M^+ peaks at m/z 741(exact mass: 741.2787; Calcd. 741.2785) along with significant fragment ion peaks at m/z 131($C_6H_5CH=CHCO$), 106(C_5H_4NCO), 105(C_6H_5CO), and 43(CH_3CO). The 1H and ^{13}C NMR(in $CDCl_3$ and pyridine- d_5) and 1H - 1H and 1H - ^{13}C COSY spectra (in pyridine- d_5) of **3** indicated the presence of partial structures A, B, and C (Fig. 1) in addition to a benzoyl, a *trans*-cinnamoyl, a nicotinoyl, two acetyl (δ_H and δ_C for CH_3 : 2.17 and 21.4; 2.31 and 21.5), three *tert*-methyl groups (δ_H and δ_C : 1.66 and 24.2; 1.63 and 23.5; 1.63 and 29.2), and four *quaternary* carbons (δ_C : 53.1, 70.8, 83.8, and 92.5). These spectral data suggest that this compound is a dihydroagarofuran derivative (**3**), having a structure analogous to ever-1 (**5**).³⁾

Fig. 1. Partial Structures in $\mathfrak{Z}$ (δ -Values in Pyridine- d_5)

As this stage, we measured the ^1H - ^{13}C long-range COSY of **3** in order to confirm the assumed structure (**3**). As expected, the ^{13}C -signals at δ 92.5(C-5) and at δ 53.1(C-10) showed long-range correlations with the ^1H -signals at δ 7.30(6-H), 5.31, 5.38(15- H_2), 2.63(7-H), 1.85(3-H), and 1.63(14- H_3) and at δ 7.30(6-H), 6.19(8-H), 6.33(9-H), 5.99(1-H), 5.31, 5.38(15- H_2), and 2.05(2-H), respectively (Fig. 2). In turn, the ^{13}C signal at δ 70.9(C-4) was correlated with the ^1H -signals at δ 2.38, 1.85(3- H_2), 2.05(2-H), and 1.63(14- H_3), and the signal at δ 53.8(C-7) with the ^1H -signals at δ 1.66(12- H_3) and 1.63(13- H_3). Also some other significant long-range correlations are shown by arrows in formula in Fig. 2.

Turning now to the location of the ester groupings, the carbonyl ^{13}C signal at δ 165.25

Fig. 2. ^1H - ^{13}C Long-Range COSY Spectra of **3** in Pyridine- d_5

a) High field region, b) Low field region, (sample: 15 mg, 20°C , 12 h run, $J_{\text{CH}}=10$ Hz).

Open circle indicate significant but weak peaks at this threshold level.

Abbreviation; Ac: acetyl, Bz: benzoyl, Cn: cinnamoyl, Nc: nicotinoyl.

showed long range correlations with the ^1H -signals at δ 6.19(8-H), 8.99 and 9.71(nicotinoyl 2'- and 4'-H), and the signals at δ 165.32 and δ 166.00 were correlated with the ^1H -signals at δ 6.33(9-H) and 8.16(benzoyl 2''- and 6''-H) and at δ 5.99(1-H), 6.04 and 7.58(cinnamoyl 7'''- and 8'''-H), respectively. On the other hand, the carbonyl ^{13}C -signals at δ 170.35 and at δ 170.74 were correlated with the ^1H -signals at δ 7.36(6-H) and 2.13(COCH_3) and at δ 5.31, 5.38(15- H_2) and 2.31(COCH_3), respectively. Thus the planar structure of **3** was proved.

The relative stereochemistry of **3** was determined on the basis of the coupling constants of each proton and the results of NOE experiments (200 MHz, CDCl_3). Irradiation at the 14-methyl group enhanced the signal intensity of 6- and 15 protons (8 and 6%, respectively) and irradiation at the 1-methine proton enhanced the signal intensity of the 9-proton (10%). Also, irradiation at the 12- and 13-methyl groups gave NOE enhancement of the 8- and 9-protons and the 7-proton, respectively. Therefore the structure of triptofordinine A-1 was proved to be **3**.

Triptofordinine A-2 (**4**), $\text{C}_{41}\text{H}_{43}\text{NO}_{12}$, mp 94-95°C, $[\alpha]_D -101.0^\circ$ (MeOH), contains a benzoyl, a *cis*-cinnamoyl, a nicotinoyl, and two acetyl residues as indicated by the MS, ^1H and ^{13}C NMR data. The ^1H and ^{13}C NMR spectra are almost identical with those of **3** except for the signals due to the olefinic protons in the cinnamoyl residue [**3**: δ_{H} 5.72, 7.28(each d, $J=16$ Hz), **4**: δ_{H} 5.21, 6.43(each d, $J=12.5$ Hz), in CDCl_3 , 200 MHz]. From this comparison of ^1H NMR, it was concluded that in the structure of **4**, the *trans*-cinnamoyl residue of **3** was replaced by the *cis*-cinnamoyl residue at C-1.

The structure elucidation of triptofordinine A-3, B, and C is in progress.

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