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Structures of Scuterivulactone C_1 and C_2 by Two-Dimensional NMR Spectroscopy. New Clerodane Type Diterpenoids from Scutellaria rivularis WALL.

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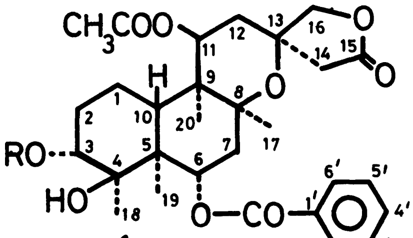
Two new clerodane type diterpenoid lactones, scuterivulactone C_1 and C_2, have been isolated from the whole herbs of Scutellaria rivularis WALL. and their structures have been elucidated by means of 2-D NMR spectroscopy including INADEQUATE and ^1H-^{13}C long-range COSY.

The ether extract from a Chinese crude drug "Ban Zhi Lian" (dried whole herbs of Scutellaria rivularis WALL., Labiatae) was separated by silica gel column chromatography followed by repeated recrystallization to give five new clerodane type diterpenoid lactones, named scuterivulactone A, B, C_1, C_2 (isolated as the acetate) and D, together with fourteen flavonoid constituents.[1)] This paper deals with the structure elucidation of scuterivulactone C_1 (1a) and C_2 (2a).



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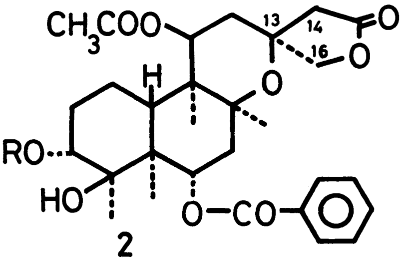

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(a: R=H, b: R=Ac)

Scuterivulactone C_1 (1a), $C_{29}H_{38}O_9$, colorless prisms (from acetone), mp 268-272 °C, $[\alpha]_D$ -7.0° (MeOH), showed absorption bands at 3580 (OH), 1786 (γ-lactone), 1725_{sh}, 1716 (ester), 1600, and 1580 cm^{-1} (phenyl) in the IR spectrum. The FABMS exhibited an ion peak due to $(M+H)^+$ at m/z 531 and the EIMS showed fragment ion peaks

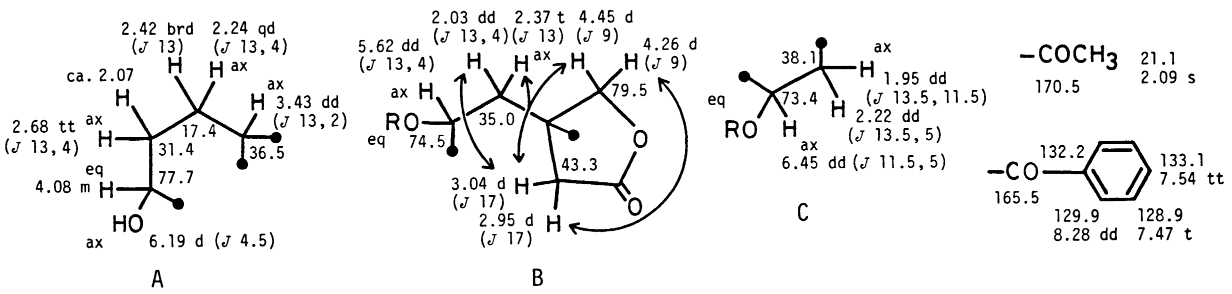

Fig. 1. Partial structures in 1a (δ-Values in pyridine-d_5).
(↷ Long-range coupling in ^1H-^1H COSY)

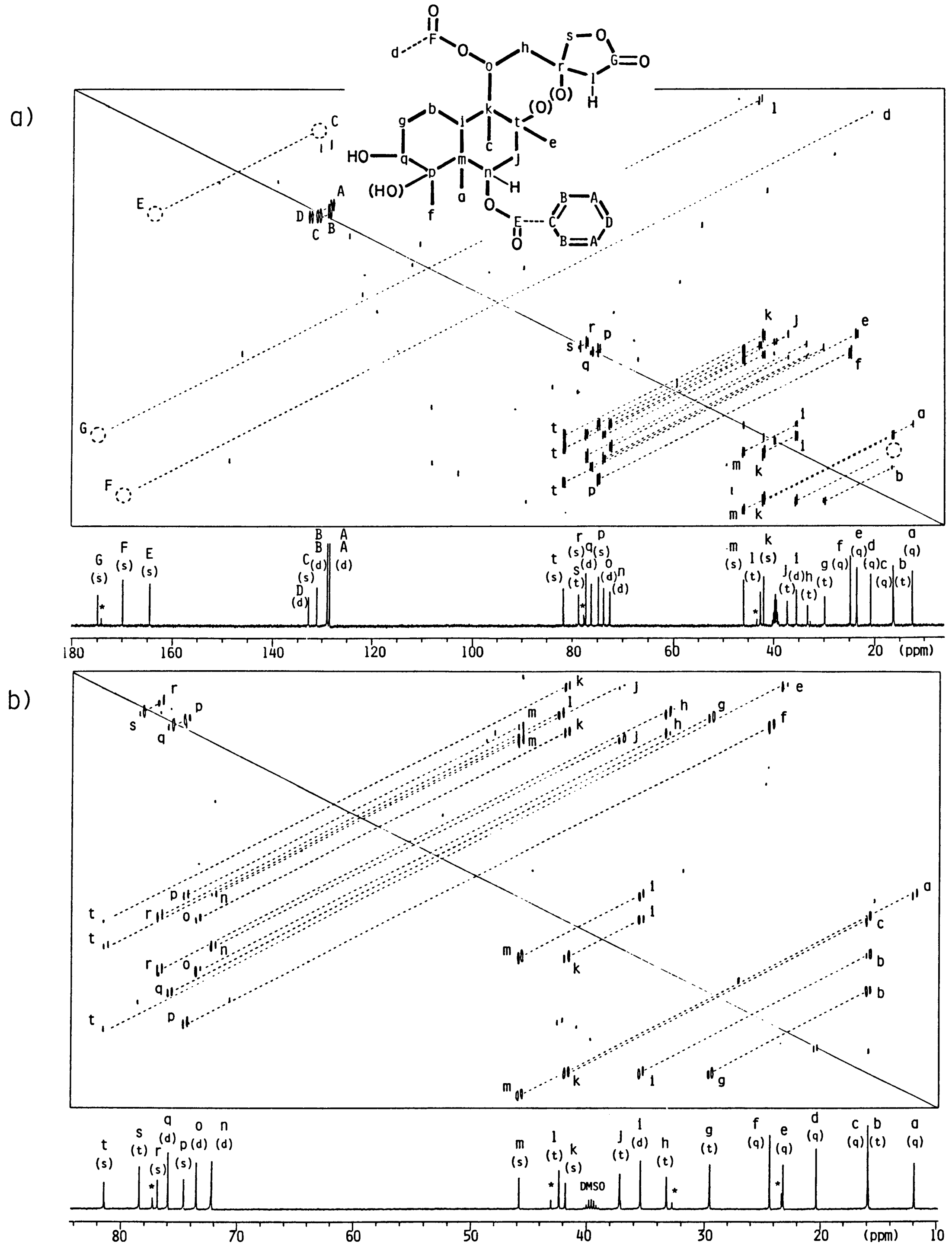

Fig. 2. 2-D INADEQUATE spectra of scuterivulactone C_1 in DMSO-d_6: a) Whole region (50 °C, 60 h run, J_{CC} = 45 Hz), b) High field region (100 °C, 40 h run, J_{CC} = 40 Hz). The spectra were measured on a JEOL GX-400 spectrometer, using 150 mg of the sample (a roughly 85:15 mixture of **1a** and **2a**) in DMSO-d_6. The ^{13}C spectrum runs along the lower axis; sp^2 carbons are marked with A - G and sp^3 carbons with a - t in the order of increasing δ values. Signals marked with asterisks are due to **2a**. The multiplicities of carbon signals were determined by the off-resonance and DEPT methods. Dotted circles indicate the expected carbon signals, which were not observed.

at m/z 512, 494, 390, 122, and 105. The ^{1}H- and ^{13}C-NMR and ^{1}H-^{1}H COSY spectra$^{2)}$ (in pyridine-d_5 and DMSO-d_6) suggested the presence of partial structures A, B, and C (Fig. 1) in addition to an acetyl, a benzoyl, a t-hydroxyl (δH 6.05), four t-methyl groups (δC 13.4, 17.0, 23.9, and 26.0; δH 1.86, 1.13, 1.29, and 1.80) and four quarternary carbons (δC 42.9, 47.2, 75.9, and 82.5). Each carbon signal except for quarternary one was assigned based on the ^{1}H-^{13}C COSY spectral data.$^{2)}$

Then, the 2-D INADEQUATE spectra$^{2)}$ of 1a were measured to clarify the sequence of carbon atoms in the molecule. The pulse system employed was (90°)-τ-(180°)-τ-(90°)-t_1/2-(135°)-t_1/2-t_2 sequence and the results were reproduced in Fig. 2, which exhibited the correlated peaks of all the coupled ^{13}C-^{13}C pairs except those between the carbons C and E, and F and d.

Thus, we applied the ^{1}H-^{13}C long-range COSY to 1a in order to confirm the connectivities of those carbons C, E, F, and d. As shown in Fig. 3, the ^{13}C-signal at δ 169.9 (F) showed long-range correlations with the ^{1}H-signals at δ 5.28 (11-H) and 2.05 (CH_3COO), while the signal at δ 164.4 (E) with those at δ 5.72 (6-H) and 7.92 (2', 6'-H_2). Also, long-range correlations between the carbon G (δ 175.1) and the 14- and 16-protons were observed. Therefore, the gross structure of scuterivulactone C_1 was proved to be as indicated in 1a.

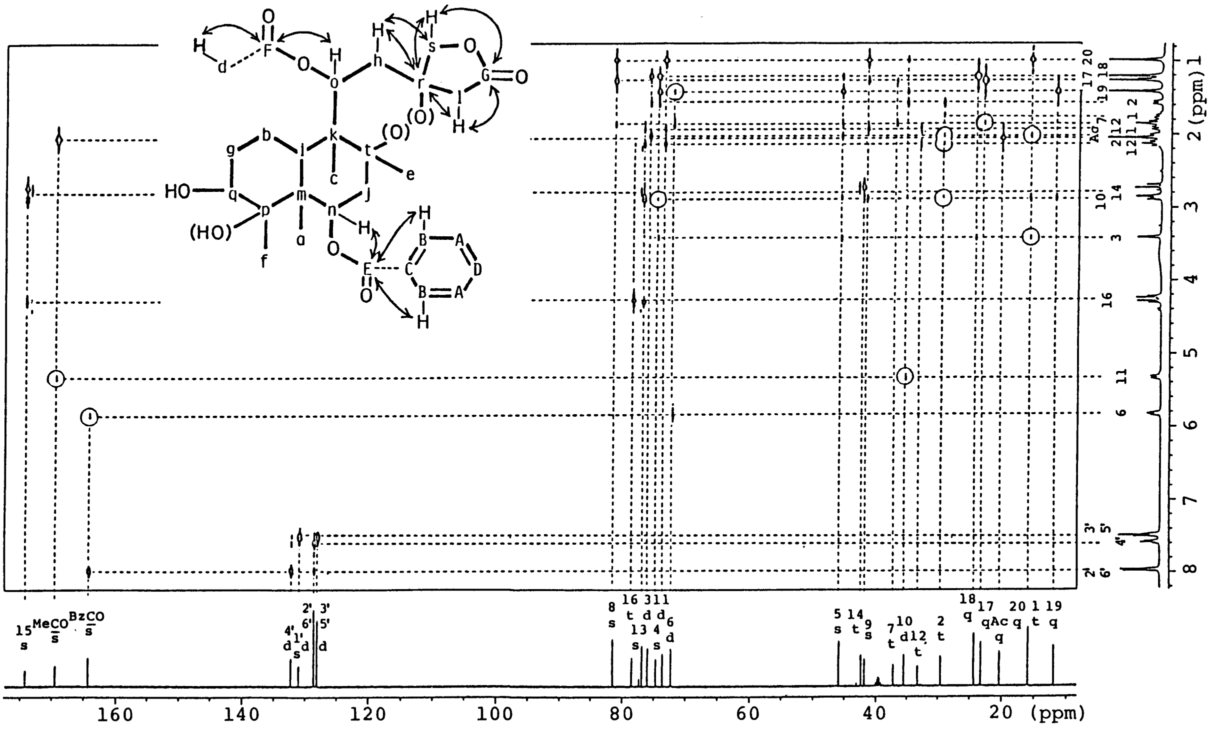

Fig. 3. ^{1}H-^{13}C Long-range shift correlation spectrum of scuterivulactone C_1 (1a) in DMSO-d_6 (sample: 150 mg, 100 °C, 12 h run, J_{CH} = 12 Hz).

The relative stereochemistry of 1a was determined on the basis of the coupling constants of each proton and the results of NOE experiments using the corresponding acetate (1b), mp 256-263 °C, $[\alpha]_D$ -50.6° ($CHCl_3$). Irradiation at the 20-H_3 and the 18-H_3 enhanced the signal intensity of the 7-, 11-, 17-, and 19-protons and the 3- and 19-protons, respectively, and irradiation at the 17-H_3 and 19-H_3 enhanced the signal intensity of the 7-, 11-, 14-, and 20-protons and the 1α-, 7-, 18-, and 20-protons, respec-

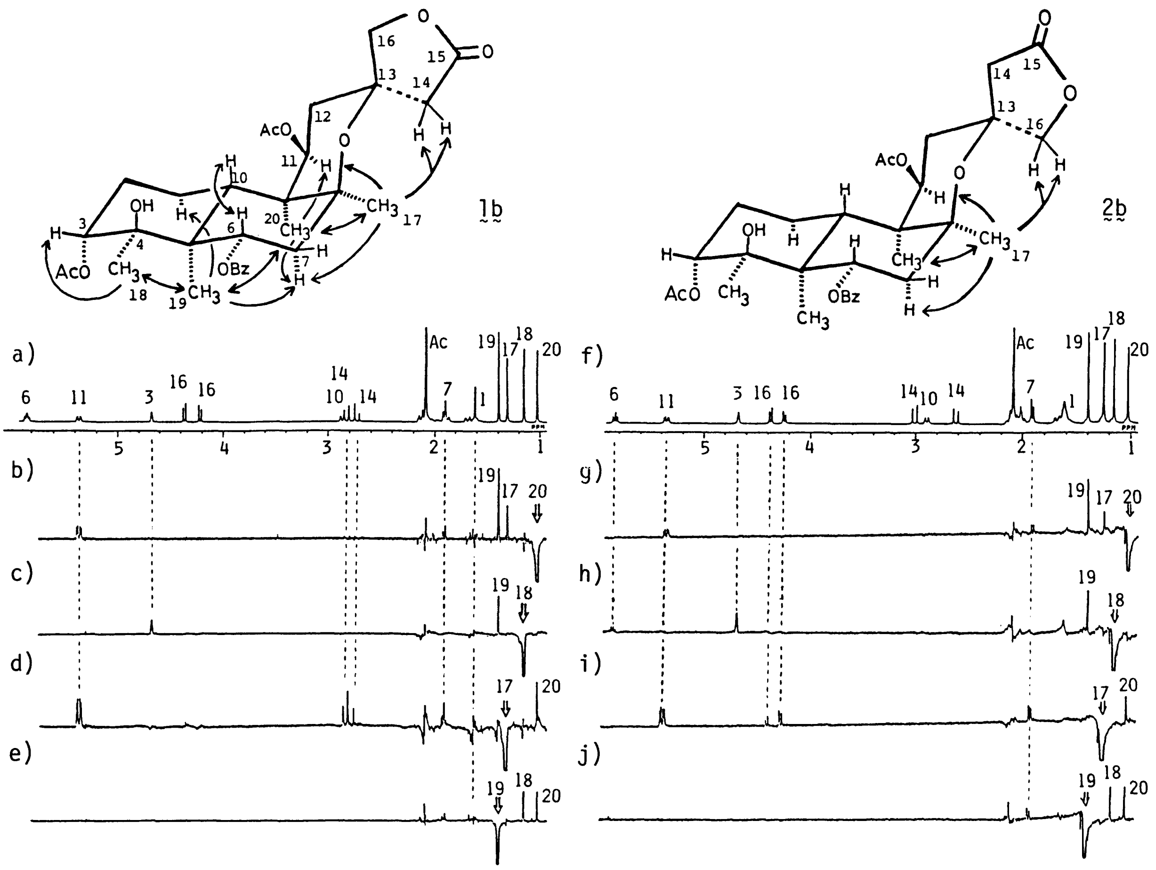

Fig. 4. Normal ^{1}H-NMR and NOE difference spectra of scuterivulactone C_1 acetate (1b) (a - e) and scuterivulactone C_2 acetate (2b) (f - j) in $CDCl_3$.

tively (Fig. 4, b - e). Also NOE's between the 6-H and the 10-H were observed.

Scuterivulactone C_2 was isolated as the acetate (2b), amorphous, $[\alpha]_D$ -31° ($CHCl_3$), from a mixture of 1a and 2a by usual acetylation followed by HPLC separation [column: TSK-GEL Silica-150, solvent: $CHCl_3$-MeOH (99.5 : 0.5)]. The acetate (2b) was considered to be the 13-epimer of 1b from comparisons of its ^{1}H- and ^{13}C-NMR spectral data with those of 1b. This was supported by the NOE experiments, in which NOE's were evidently observed between the 17-H_3 and the 16-H_2 as well as between the 17-H_3 and the 11-H, as shown in Fig. 4, g - j.

These findings led us to conclude that the structures of scuterivulactone C_1 and C_2 should be represented by the formula 1a and 2a, respectively.

Absolute configurations of 1a and 2a and structures of scuterivulactone A, B, and D are currently under investigation.

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