Reinvestigation of the Structure of Galbanic Acid by 2D NMR Techniques Including 2D INADEQUATE

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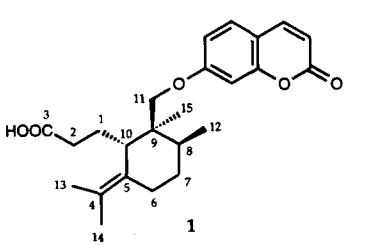
The galbanic acid (**1**) is a sesquiterpene coumarin ether occurred in the resin of *Ferula assa-foetida* L. (Umbelliferae) and also found in various species of the *Ferula genus*. It had been isolated for the first time from the gum resin of *F. microloba* in 1972[1] and its structure (**2**) was postulated by Borisov *et al.* in 1973.[2] However, the proposed structure was completely revised as **1** in 1980 by an analysis of 300 MHz ^{1}H NMR spectrum.[3] On the other hand, Kajimoto and his co-worker had isolated a compound named asacoumarin B (**3**) from *F. assa-foetida in 1989*, and suggested a structure which had been quite different from that of **1**.[4] They had proposed the structure of asacoumarin B particularly by the investigation of the ozonolysis product. However, the asacoumarin B was revealed recently to be a galbanic acid (**1**) by Appendino *et al.*[5] They had investigated the structure of **3** by the aid of 2-D NMR techniques, which was not depicted in detail in literature, and confirmed the structure of galbanic acid as **1** suggested by Bagirov *et al.* in 1980.[3]

In the process of searching for new compounds from the gum resin of *F. assafoetida*, we found the presumed same compound as **1** at least on the basis of the patterns and chemical shifts of ^{1}H and ^{13}C NMR. Although there are numerous indirect ways[6] to elucidate the carbon skeleton of organic molecules, indirect approach fails and some ambiguities remain further in the assignment of carbon resonances when the proton spectra do not exhibit well resolved signals. All of these limitations could be solved if we could measure the direct carbon-carbon correlations.

Because the importance of correct chemical shifts assignments even can never be overstated to unlock all the information present in the spectrum, it is worth reinvestigating the controversial structure of galbanic acid (**1**).

Consequently, we reinvestigate the structure of **1** by 2D NMR techniques including 2D INADEQUATE and herein report the unambiguous results and correct stereochemistry of **1**.



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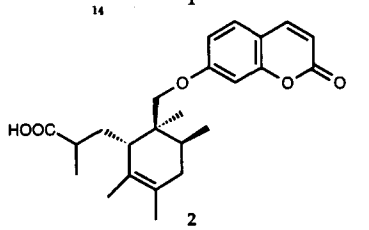

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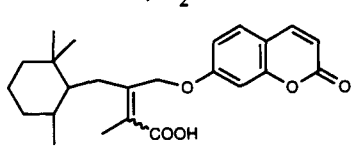

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Experimental

Isolation of galbanic acid. The gum resin of *F. assafoetida* was obtained from Chien Yuen Herbal Medicinal Co., Taipei, Taiwan and was identified by the Herbarium of Natural Products Research Institute, Seoul National University, Korea, where the voucher specimen is deposited.

The gum resin of *F. assafoetida* (1.2 kg) was refluxed with MeOH for 4 hours. The extract was collected and concentrated to dryness to give a crude extract (*ca.* 460 g), which was suspended in water and subjected to partition with hexane (56 g), CH_2Cl_2 (290 g) and *n*-butanol (50 g), successively. Five grams of CH_2Cl_2 soluble part was purified by silica gel column chromatography and eluted with hexane and EtOAc mixture by gradient manner (R_f=0.3, hexane/EtOAc=3 : 1) to give *ca.* 150 mg of galbanic acid, which was further purified by preparative TLC on silica gel.

NMR Measurements. All NMR spectra were recorded on Bruker AMX-500 spectrometer. In all cases, a 5 mm Willmad NMR tube was used and 80-90 mg of galbanic acid (**1**) was dissolved in 0.5 mL of $CDCl_3$.

Proton and carbon chemical shifts are reported in ppm relative to TMS, but were measured against the central solvent peak at 7.24 and 77.00 ppm, respectively. The ^{1}H spectrum was collected as 32 K data points over an 7500 Hz spectral width using a 30 °C pulse (Normal 1D) and data were processed using exponential multiplication with a 1.5 Hz line broadening.

The ^{1}H-^{1}H COSY spectrum was obtained using COSY-45 pulse sequence. The spectral widths were both set at 7500 Hz. An initial matrix of 1 K (256 data points was zero-filled to give 1 K × 1 K and was processed by sinusoidal multiplication in each dimension followed by symmetrization of the final data matrix. Eight transients with a 1 s delay were accumulated for each of the 256 increments. The total acquisition time was 40 min.

The long range COSY was obtained by the cosylr program with the 0.12 s dalay for evolution of long range couplings. For each increment, 32 scans were accumulated and the total acquisition time was 3 h.

The two dimensional NOESY spectrum in phase-sensitive mode was recorded using the noesytp program, where the mixing time and the relaxation delay time used were 0.3 s and 1 s, respectively. Scans were accumulated for each of

the 512 increments, and the total acquisition time was 6.5 h. The collected 1 K × 1 K was processed by squared sine-bell window shifted by π/2 in both dimensions.

In the heteronuclear correlation (HETCOR) experiments, the spectral widths were 35 KHz in F2 and 7.5 KHz in F1. The initial matrix of 1 K × 128 was zero-filled to 2 K × 256 to give the final spectrum. For each increment, 16 transients were accumulated; the relaxation delay was 1 s and the total acquisition time was 40 min.

The long-range ^{1}H-^{13}C HETCOR spectra were obtained with the parameter optimized by J_{C-H}=4, 6, and 9 Hz, respectively. Other acquisition parameters were the same as in the normal HETCOR experiment except that for each increment 256, 192, and 128 scans respectively; the total acquisition time for each experiment was 13, 9, and 6 h, respectively.

In the 2D INADEQUATE experiment, the spectral width of F1 dimension was the half of the F2=22730 Hz to increase the digital resolution of F1 dimension (*vide infra*). The initial matrix of 4 K × 242 was zero-filled to 4 K × 256 to give the final spectrum and was processed by exponential multiplication (LB=2.5) in F2 dimension and π/2 shifted sine-bell window in F1 dimension. For each increment, 512 transients were accumulated; the relaxation delay was 2 s and the ^{13}C-^{13}C coupling constant was set to 45 Hz and the total acquisition time was 74 h.

Results and Discussion

High Resolution EIMS revealed the formula $C_{24}H_{30}O_5$ (m/z 398.2130, 3.7 mmu error) for **1**. A combination of DEPT[7] and HETCOR experiments[8] of **1** confirmed that there were four CH_3 groups, five CH_2 groups, seven CH groups, and eight quaternary carbons in the molecule. A large carbon peak corresponding to two carbons at δ 20.06 was found to be two methyl carbons linked to δ 1.56 and δ 1.39 methyl protons in HETCOR spectrum, respectively. The remaining one proton was concluded to be a carboxylic exchangeable proton and observed as a broad signal at δ 9.8-8.8.

Without the aid of ^{13}C-^{13}C connectivity experiment, the presence of a coumarin moiety was easily confirmed by the proton COSY, long-range COSY, and HETCOR experiments. The results of long-range HETCOR correlations of nine ^{13}C chemical signals were in good agreement with the coumarin moiety. However, the remaining moiety of the molecule could not be established by the usual ^{1}H-^{1}H and ^{13}C-^{1}H connectivity because of the incomplete analysis of proton spectrum in which the signals at δ 1.9-1.8 consist of four protons; one methine proton, one of methylene protons, and two of other methylene protons.

Therefore, we first determined direct ^{13}C-^{13}C connectivities by using two-dimensional INADEQUATE experiment.[9] The pulse sequence employed was the 90°-τ-180°-τ-90°-t_1-90°-t_2 with quadrature detection in F1 dimension.

The partial carbon-13 2D INADEQUATE spectrum of **1** obtained by the condition of F2=2F1 (folded twice) is shown in the Figure 1. Beginning with the carbon resonating at 71.53 ppm (Figure 1), we can easily identify that this peak is responsible for the carbon 11 referring to the multiplicities of carbon spectrum determined by DEPT experiment[8] and proton chemical shift determined by C-H HETCOR experiment.[9] This methylene carbon has a correlation with the quaternary carbon C-9 at 40.59 ppm. Then this quaternary carbon C-9 has additional correlations peaks with two methine carbons at C-8 (δ, 34.68) and C-10 (δ, 42.47), and methyl carbon at C-15 (δ, 22.35). The methine carbon C-8 has a correlation peak with the C-7 resonating at 31.79 ppm which has an additional correlation peak with the C-6 at 24.32 ppm. Then both C-6 and C-10 have a correlation peak with the C-5 at 129.36 ppm. By doing this way, all the correlations are drawn parallel to F2 axis as shown in the Figure 1 except those between the C-2 and C-3 and between the C-4 and C-5.

The strongly coupled AB connection between the C-4 and C-5 was confirmed by the long range C-H correlation peaks between the C-5 and methyl protons at C-14 and the C-4 and the methine protons at C-10 and one of methylene proton (6α) at C-6.

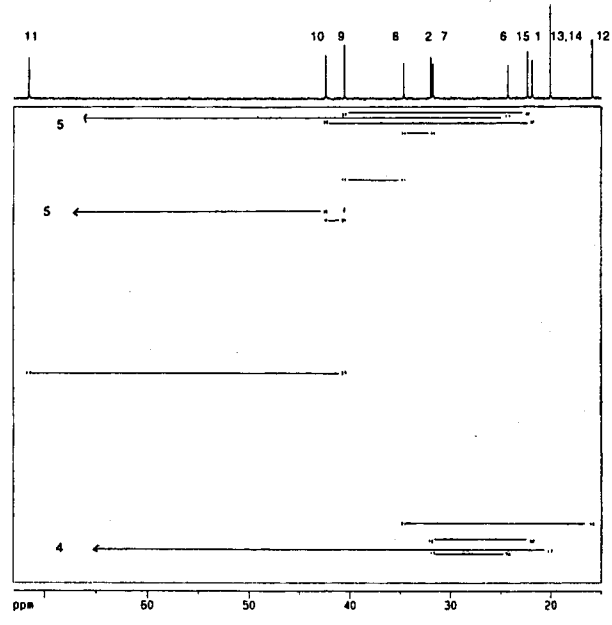

Figure 1. Partial 2D-INADEQUATE spectrum of 1.

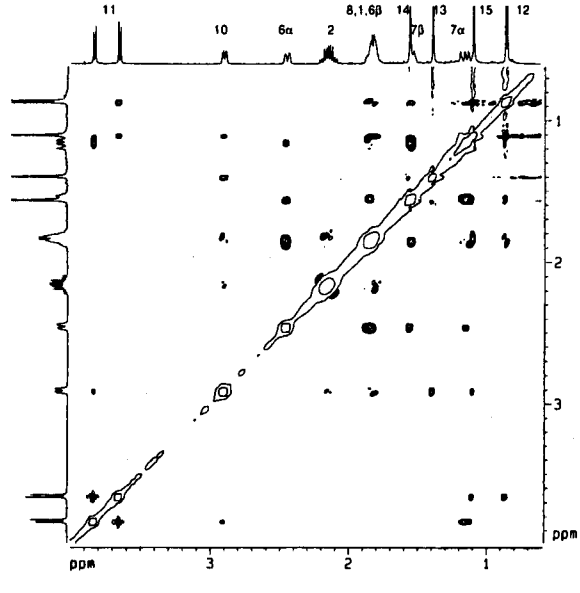

Figure 2. 2D-NOESY spectrum of 1.

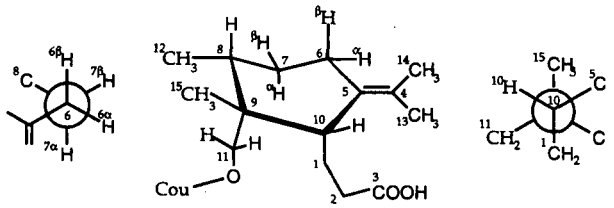

Figure 3. Revised configuration of galbanic acid.

The carboxylic group was also confirmed by the long range C-H correlation peaks between the carbonyl carbon 3 at 180.02 ppm and the methylene protons at C-2.

With this carbon fragment information and the C-H HETCOR experiment, the phase sensitive NOESY experiment was done for stereochemical analysis. The expanded partial NOESY spectrum of **1** is shown in Figure 2. Based on the fact that the carbons at C-4, C-5, C-6, C-10, C-13, and C-14 are in the same plane, we may easily predict the NOE cross peaks between protons at C-13 and C-10 or C-1.

Figure 2 shows clearly the NOE cross peak between the protons at C-10 and C-13 which says that the previous chemical shift assignments of methyl protons at C-13 and C-14 are reversed.[5] On the other hand, the methyl protons at C-14 show the NOE peak with the α-proton at C-6. Thus, the NOE results of the methyl protons at C-13 and C-14 not only can correct the previous chemical shift assignments of these protons, but can support the pseudo-equatorial position of the protons at C-10 and H-6α.

With these fixed two positions and the coupling constants (J) 13.7, 13.0, 13.0 and 4.7 Hz in *dddd* splitting pattern of H-7α, we can arrange the stereochemistry of H-6β and H-8 to have anti-parallel axial positions with respect to H-7α, respectively. The further streochemical arrangements in C-8 and C-9 are supported by the NOE peaks between the H-7α and one proton at C-11, and between the protons at C-12 and the other proton at C-11. Thus, the two methyl groups, which were assigned as α and β previously, at C-8 and C-9 should have α and β positions in the twisted boat conformation, respectively. The revised configuration of **1** is shown in Figure 3 with partial conformations. The final assignments of chemical shifts of the α and β protons at C-6 and C-7, which belong to equatorial or axial protons of cyclohexane depending on position, are valid for relationship, $\delta H_a < \delta H_e$.

In summary, we have shown that unambiguous structural analysis of galbanic acid (**1**) was done by using modern 2D NMR techniques including the 2D INADEQUATE experiment. It is also evident that the stereochemistry at C-8 and C-9 determined previously should be revised.

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Enantioselective Synthesis of the C20-C25 Portion of the Cytotoxic Natural Product, Amphidinolide B1

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Amphidinolides A-Q have recently been isolated from dinoflagellates, genus *Amphidinium*, symbiotic with the Okinawan marine flatworms and generally exhibited potent toxicities against cancer tumor cell lines.[1,2] Although the chemical structures of them were elucidated mainly on the basis of extensive spectroscopic studies including 2D NMR experiments, their configurations still remain unclear except for amphidinolides B, J, and L. The relative stereochemical relationship of amphidinolide B group (**1**), the most representative and important group of amphidinolides family, was unambiguously solved by single crystal X-ray diffraction method[3a] and the absolute configuration of amphidinolide B1 (**1a**) was established on the basis of enantiospecific synthesis of a degradation product.[3b] The absolute configurations of amphidinolide J and L were determined similarly.[4a,4b]

In our program toward the total synthesis of amphidinolide B1 (**1a**), the target molecule was initially divided into three components **2a**, **2b**, and **2c** and enantioselective