Communications to the Editor

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^{13}C NMR SPECTRAL STUDIES OF ENTAGENIC ACID TO ESTABLISH ITS STRUCTURE

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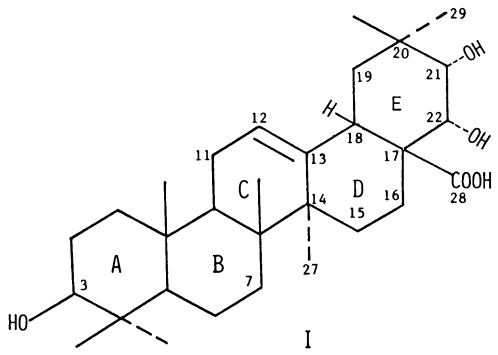
The structure of entagenic acid , a sapogenin of the saponin isolated from the bark and seed kernel of Entada phaseoloides Merrill (Leguminosae), has been established as 3β,15α,16α-trihydroxy-18β-olean-12-en-28-oic acid by 2D-NMR spectroscopy using the INADEQUATE method.

KEYWORDS——Entada phaseoloides; entada saponin; entagenic acid; Leguminosae; 2D-INADEQUATE

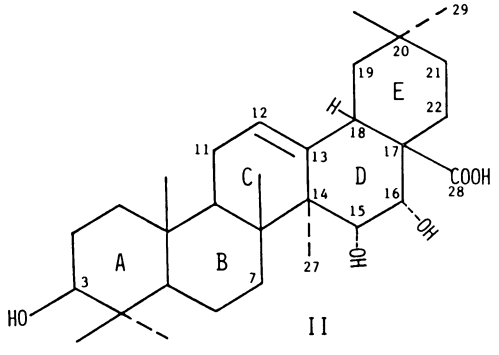
Previously we isolated a saponin named entadasaponin (ES)-IV from the bark of Entada phaseoloides Merrill (Leguminosae) along with some other homologous saponins, ES-II and ES-III. These saponins all have the same sugar components attached to a monoterpene and an acetyl group, and differ only in the sapogenins, entagenic acid, oleanolic acid and echinocystic acid.[1,2] A saponin of entagenic acid with a different sugar component has also been isolated from the seed kernel of the same plant.

The structure of entagenic acid was first studied by Barua as a new triterpene of Entada seed.[3] He proposed two alternative structures, 3,21,22-trihydroxy-18β-olean-12-en-28-oic acid (I) and 3,15,16-trihydroxy-18β-olean-12-en-28-oic acid (II). Later, he presented the first formula (I) as the most probable structure of entagenic acid,[4,5] but this has been changed to (II) by some chemical evidence.[6]

Since the formula (I) had been referred to entagenic acid as before by several authors,[7,8,9] we stated in our previous paper[2] that formula (I) represents entagenic acid as the sapogenin of ES-IV.



I

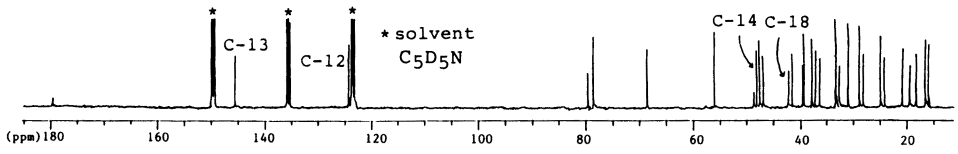


II

Although carbon signals due to the A-, B- and C-rings were reasonably assigned to almost all the ^{13}C NMR signals of entagenic acid formulated as (I), eight signals at δ 31.0, 32.8, 36.4, 37.2, 48.2, 48.8, 68.6 and 79.7 could not be assigned correctly to C-7, -14, -15, -16, -17, -20, -21 or -22, respectively. If two hydroxyls of the α-glycol system are attached to the C-21α and C-22α positions as formulated in (I), two carbon signals at δ 68.6 and 79.7 may be assigned to C-21 or C-22, and then two signals at δ 32.8 and 36.4 would be assigned to C-15 or C-16. In that case, a signal at δ 31.0 due to the C-20 position should be shifted to a somewhat lower field by the substitution effect of C-21 and C-22 hydroxyls, but the C-20 carbon signal of entagenic acid appears, as above, at the ordinary position given by the oleanan-type triterpenes without any functional groups in the E-ring. Besides, the hydroxyl group would cause the shift in the carbon signal of the C-29α methyl group. Therefore, the presence of the α-glycol system at C-21 and C-22 on the E-ring has been excluded. Alternatively the α-glycol system must exist at C-15 and C-16. The signals at δ 68.6 and 79.7 could be assigned to C-15 and C-16, and those at δ 36.4 and 32.8 to C-21 and C-22. All the remaining signals including δ 31.0 attributable to C-20, δ 78.6 to C-3, δ 37.2 to C-7 and δ 20.7 to methyl carbon at C-27 are reasonably assigned to support the structure (II) for entagenic acid. (Table I)

For the unequivocal establishment of the structure, 2D NMR spectroscopy has been applied to entagenic acid using the Incredible Natural Abandance Double Quantum Transfer Experiment (INADEQUATE) method.[10] As the spin-spin coupling between ^{13}C nuclei is observed by this method, all the networks of C-C linkages in an organic molecule could be established. By analyzing of the 2D-INADEQUATE spectrum (Fig.1-1,1-2), the correlation of the carbon signals (C-12, 13, 14, 15, 16, 17 and 18) was clarified. At the same time, all the carbon atoms and their connections in the entagenic acid molecule were clealy assigned to prove the formula (II).

A low field shift of the C-7 carbon signal and an upfield shift of the C-27 methyl signal would be caused by the effect of α-glycol system at C-15α and C-16α. Consequently the structure of entagenic acid has been established as 3β,15α,16α-trihydroxy-18β-olean-12-en-28-oic acid (II).



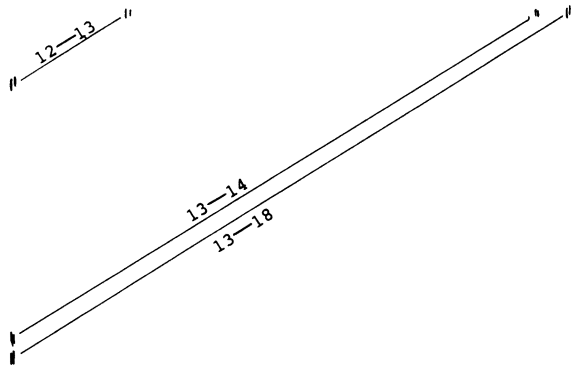


Fig. 1-1. 2D-INADEQUATE Spectrum of Entagenic Acid, Sample 130 mg/0.5 ml, 14 Hours

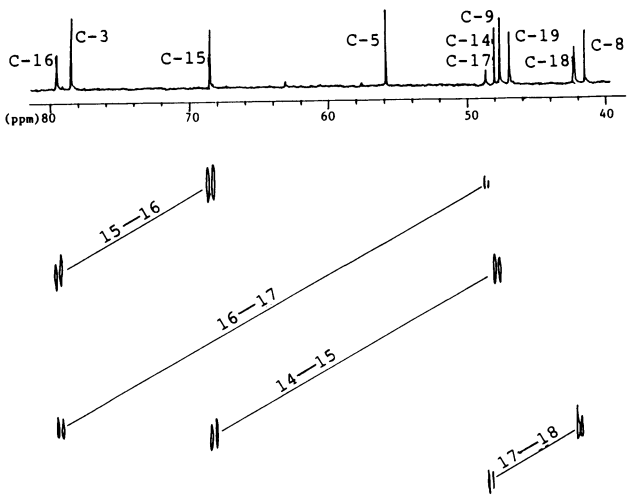


Fig. 1-2. 40-80 ppm Region of Fig. 1-1

In order to simplify the complicated spectrum, only the pertinent correlated peaks are shown in Fig. 1-1 and Fig. 1-2. The spectrum was assigned on the basis of correlated signals at δ 124.3 and 145.8 (C-12 and C-13), which were easily distinguish from others.

Table I. ^{13}C NMR Chemical Shift Values (ppm) of II

Carbon No	ppm	Multiplicity
1	39.5	t
2	28.3	t
3	78.6	d
4	39.4	s
5	56.0	d
6	19.4	t
7	37.2	t
8	41.6	s
9	47.8	d
10	37.9	s
11	24.2	t
12	124.3	d
13	145.8	s
14	48.2	s
15	68.6	d
16	79.7	d
17	48.8	s
18	42.4	d
19	47.1	t
20	31.0	s
21	36.4	t
22	32.8	t
23	28.9	q
24	16.5	q
25	15.9	q
26	18.3	q
27	20.7	q
28	180.0	s
29	33.4	q
30	25.0	q

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