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SHORT COMMUNICATION

A CRYSTALLINE SAPONIN WITH ANTI-TUMOR ACTIVITY FROM *ENTADA PHASEOLOIDES*

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Abstract—A crystalline saponin, isolated from the seed kernels of *Entada phaseoloides*, has the tentative empirical formula $C_{45}H_{82}O_{27}$ Acid hydrolysis yeilds a crystalline sapogenin $C_{30}H_{48}O_5$ which appears to be identical with entagenic acid, together with arabinose and xylose The saponin shows significant activity against Walker 256 carcinosarcoma in rats

DURING the course of screening plants for anti-tumor substances, it was observed that methanolic extracts of the seed kernels of *Entada phaseoloides* Merril Syn. (Leguminosae) showed consistent activity in the Walker 256 carcinosarcoma system in rats. The isolation and characterization of the active principle are described in this report.

Preliminary fractionation by countercurrent distribution and gel-filtration through Sephadex columns gave an active fraction which exhibited marked surface active properties, hemolysed erythrocytes and gave positive anthrone and Liebermann–Burchard tests. Further purification was carried out using partition chromatography on silicic acid The sample thus purified was obtained as a crystalline solid when solutions in aqueous butanol were concentrated The crystalline saponin formed colourless needles, m.p 223–225° and appeared homogeneous in TLC. Elemental analysis suggested an empirical formula $C_{45}H_{82}O_{27}$. Assignment of an exact molecular formula must await a more detailed structural information.

The crystalline sample showed all the expected characteristics of a saponin It showed only end absorption in its UV spectrum Its IR spectrum gave evidence for the presence of hydroxyl (3400, 1160, 1140–980 cm^{-1}), carbonyl (1730, 1245 cm^{-1}), and methyl groups (1450, 1370 cm^{-1}) and unsaturation (1640 cm^{-1}) among others.

No reports on the isolation of a saponin from *Entada phaseoloides* have appeared so far However, by hydrolysis of the saponin-containing fraction, the sapogenin, entagenic acid was isolated and characterized [1,2] From a related plant, *Entada scandens*, isolation of two saponins was described but details on characteristics are not sufficient for proper comparison.[3]

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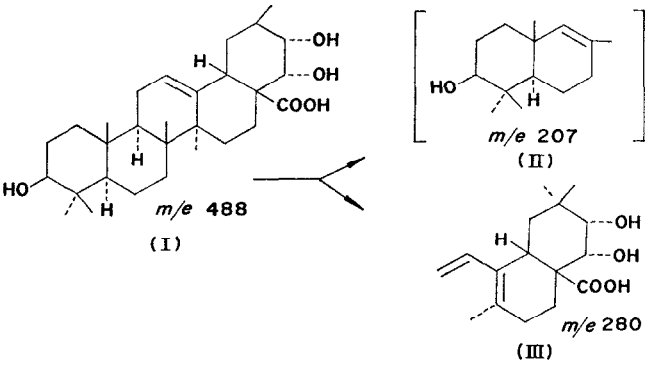
[1] A K BARUA, *Naturwissenschaften* **43**, 250 (1956)
[2] A K BARUA, *Tetrahedron* **23**, 1499 (1967)
[3] N L DUTTA, *J Sci Ind Res India* **15B**, 194 (1956)

Acid hydrolysis of the saponin gave a crystalline aglycone of composition $C_{30}H_{48}O_5$ Its physical and spectral properties suggested its possible identity with entagenic acid (I) The mass spectrum showed the molecular ion at *m/e* 488 and the fragmentation pattern was also consistent with the proposed structure of entagenic acid.[2] The abundance of the fragments at *m/e* 280 and 207 strongly favor the retro Diels–Alder pathway to yield the two



primary fission products (II) and (III). The aglycone was also converted to the methyl ester and its analysis, m p and IR spectral data were in agreement with those reported for methyl entagenate.

The relative position of the carbonyl absorption band in the saponin (1730 cm^{-1}), in the sapogenin (1695 cm^{-1}) and its methyl ester (1705 cm^{-1}) suggest the possibility that the sapogenin and the sugar residue might be joined by an ester linkage In addition to the crystalline aglycone, paper chromatography of the hydrolysate revealed the presence of arabinose and xylose

TABLE 1 ACTIVITY OF THE *Entada* SAPONIN IN W-256 SYSTEM

Dose (mg/kg)	Number survived	Relative change in body weight	Tumor inhibition (%)
45	2/6	−2 5	80
30	6/6	+0 1	82
20	6/6	+0 2	76
14	6/6	+2 3	76
45	4/6	−2 2	96
30	6/6	−0 2	100
20	6/6	+0 3	89
14	6/6	+2 8	70

The authors are indebted to Dr T J McBride, of the John L Smith Memorial Cancer Research for these results The protocol used in testing against Walker 256 tumor system in rats is that of *Cancer Chemotherapy Report No* 25, p 12, December 1962 The tumor cells are implanted intramuscularly in the thighs of non-inbred albino rats, 6 animals per test group Treatment is begun 3 days after implant at one dose daily for 4 days The animals are sacrificed on the seventh day The weights of tumors of the test animals are compared with those of control animals Tumor inhibition of greater than 54% is considered significant

[4] S. M KUPCHAN, M TAKASUGI, R M SMITH and P. S. STEYN, *Chem Commun* 969 (1970).

The crystalline saponin showed significant activity (Table 1) against Walker 256 carcinosarcoma in rats. It is appropriate to note that a saponin P with antitumor activity, isolated from *Acer negundo* by Kupchan *et al.*,[4] yields a sapogenin which is the 21β epimer of entagenic acid

EXPERIMENTAL

Isolation of the saponin Ground kernels of *Entada phaseoloides* (10 lb) were defatted with petrol and extracted twice with 50% aq MeOH The extracts were concentrated and the aq concentrate (20% solids) extracted three times with MeOH–*n*-BuOH (1 9) The combined extract was concentrated azeotropically and the concentrate freeze-dried

Purification was carried out by partition chromatography using a 2 1 mixture of silicic acid (Mallinckrodt 100–200 mesh) and cellulose as support and the upper and lower layers of the system *n*-BuOH–EtOAc–H_2O (1 1 1) as the mobile and stationary phases respectively After development, the fractions were tested for absorbance at 280 nm and TLC behaviour (SiO_2, *n*-BuOH–HOAc–H_2O, 12 3 5, and detection by spraying with 5% H_2SO_4 in HOAc) When the absorbance reached a minimum, the mobile phase was changed to the upper layer of *n*-BuOH–EtOAc–H_2O (2 1 1) which eluted the saponin The active fractions were concentrated, whereby the saponin separated as a crystalline solid It was filtered, washed and recrystallized from *n*-BuOH–MeOH–H_2O The saponin was readily soluble in water and lower alcohols but insoluble in acetone and ethyl acetate Anal Calc for $C_{45}H_{82}O_{27}$ C, 51 22, H, 7 83 Found C, 51 11, 51 31, H, 7 76, 7 12 The compound gave a single spot, R_f 0 3 in the system already described $[\alpha]_D^{25}$ −25 8° (MeOH) The purified saponin represented a yield of approximately 0 15% of the powdered seeds

Hydrolysis A solution of the saponin (0 5 g) in 3 N HCl (50 ml) was boiled under reflux for 2 hr After cooling, the solid was filtered and crystallized twice from MeOH It formed colourless rectangular plates, m p 310–315°, $[\alpha]_D^{25}$ +34 9° (EtOH) Anal Calc for $C_{30}H_{48}O_{50}$ H_2O C, 71 11, H, 9 94 Found C, 71 01, H, 10 04 IR(cm^{-1}), 1100, 1035 (OH), 1695, 1275, 1250 (COOH), 1630, 880, 780 ($R_1R_2C{=}CHR_3$), 1460, 1380 (CH_3)

The sapogenin (0 1 g) in EtOH was treated with a slight excess of ethereal CH_2N_2 After 6 hr, the solution was concentrated and the product crystallized from benzene–hexane The methyl ester formed colorless needles, m p 251–252° Anal Calc for $C_{31}H_{50}O_5$ C, 74 06, H, 10 02, OCH_3, 6 19 Found C, 74 05, H, 9 95, OCH_3, 6 44 IR(cm^{-1}) 3300, 1089 (OH), 1705, 1235, 1166 (CH_3COO), 1639 ($R_1R_2C{=}CR_3H$), 1450, 1380 (CH_3)

The filtrate from the sapogenin was freed from acid by the use of Amberlite IR-45 and concentrated to a glass, when examined by paper chromatography, xylose and arabinose were detected

Key Word Index—Entada phaseoloides, Leguminosae, saponin, entagenic acid, anti-tumor activity