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PHASEOLOIDIN, A HOMOGENTISIC ACID GLUCOSIDE FROM *ENTADA PHASEOLOIDES*

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Key Word Index—*Entada phaseoloides*; Leguminosae; homogentisic acid glucoside; phaseoloidin.

Abstract—The structure of phaseoloidin isolated from the seeds of *Entada phaseoloides* has been determined as homogentisic acid 2-*O*-β-D-glucopyranoside by chemical and spectral means.

INTRODUCTION

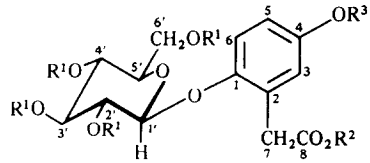
The seeds of the plant *Entada phaseoloides* Merrill are known to be poisonous to fish and are considered to be toxic, emetic and anthelmintic [1]. Barua *et al.* [2] characterized the active principles as triterpenoid saponins and they identified two known sapogenins as oleanolic and echinocystic acids besides a new sapogenin, entagenic acid. Later Wen Chih Lin *et al.* [3] isolated a glycoside of entagenic acid from the same source and reported its significant activity against Walker 256 carcinosarcoma in rats. Two new thioamides, entadamide A and B, have recently been reported from the seed kernel of this plant [4,5]. A new glycoside of entagenic acid, designated Entada saponin-III, has also been reported recently from the bark of this plant [6]. The present paper reports the isolation of a new glucoside called phaseoloidin from the seed kernel, whose structure has been established as homogentisic acid 2-*O*-β-D-glucoside (**1a**).

RESULTS AND DISCUSSION

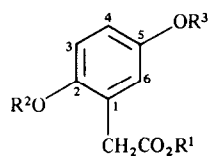
Phaseoloidin (**1a**), $C_{14}H_{18}O_9$, has been found to be a phenolic glucoside from its UV and IR spectra. This was confirmed by successful enzymatic hydrolysis with β-glucosidase, which yielded glucose, identified by paper chromatography and the aglycone (**2a**). The latter on treatment with diazomethane yielded the monomethyl ester (**2b**) as the major product. Hydrolysis of phaseoloidin with methanolic hydrochloric acid yielded the same monomethyl ester (**2b**) besides glucose. A TLC comparison of methyl 2,4-dimethoxyphenyl acetate (**3**), prepared following a procedure [7,8] reported for an isomeric compound, with the monomethyl (**2c**) and dimethyl (**2d**) derivatives of **2b**, prepared by excess diazomethane treatment of the latter, demonstrated their nonidentity. Finally, the aglycone methyl ester (**2b**) was identified as methyl homogentisate by direct comparison (mp, mmp, co-TLC, ^{1}H NMR and MS) with the methyl ester prepared by refluxing authentic homogentisic acid (**2a**) with methanolic hydrochloric acid. Homogentisic acid was earlier reported from the urine of alkaptonuric patients [9].

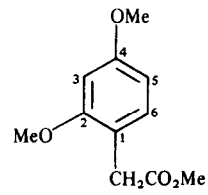
On acetylation phaseoloidin yielded a pentaacetate (**1b**). Its ^{1}H NMR and mass spectral data were found to be in conformity with the structure **1b**. On treatment with excess diazomethane phaseoloidin furnished a dimethyl derivative (**1c**) which on acetylation yielded the tetraacetate (**1d**). Its mass spectral data recorded all the peaks expected of the tetraacetyl glucosidyl moiety and the derivatised aglycone part.

The ^{1}H NMR spectrum of **1d** showed all the signals expected of the structure arrived at. Since the anomeric proton of the sugar appeared upfield of δ 5.5, the C-1′ hydroxyl is present, not as an ester, but as an acetal in **1d** [10], and this will be the case in phaseoloidin and all its derivatives. The coupling constant (8 Hz) of the anomeric proton suggested a β-glucosidic linkage, a conclusion supported by successful hydrolysis of phaseoloidin by β-glucosidase, stated earlier. The signals at δ3.56 and 3.72 (1H, *d* each, $J=16$ Hz) observed in the ^{1}H NMR spec-



1a $R^1 = R^2 = R^3 = H$
1b $R^1 = R^3 = Ac$; $R^2 = H$
1c $R^1 = H$; $R^2 = R^3 = Me$
1d $R^1 = Ac$; $R^2 = R^3 = Me$





2a $R^1 = R^2 = R^3 = H$
2b $R^1 = Me$; $R^2 = R^3 = H$
2c $R^1 = Me$; $R^2/R^3 = Me/H$
2d $R^1 = R^2 = R^3 = Me$

3

trum of **1d** could only be assigned [11] to the benzylic methylene protons. But their non-equivalence required the placement of a group bulkier than methoxyl, i.e. the glucosidyl moiety at the *ortho*-position (C-2), leaving C-5 for the aromatic methoxyl, for the signal due to the benzylic methylene protons of **3** appeared as a singlet only.

Molecular rotation calculations [12] revealed that the glucose part in phaseoloidin was D-glucose. Thus, **1a** has the [M] value of $-135.73°$ showing an acceptable difference of 69.38° from the calculated value (based on the [M] value of $-66.35°$ for methyl β-D-glucopyranoside). If glucose had the L-configuration in **1a**, the difference (based on the [M] value of $+66.35°$ for methyl β-L-glucopyranoside) would be 202.08°, which was obviously unacceptable.

Phaseoloidin is thus homogentisic acid 2-*O*-β-D-glucopyranoside (**1a**). The structure of phaseoloidin received further support from the excellent similarity in the ^{13}C NMR data of **1a** and salireposide (**4**), reported recently [13], as shown in Table 1. Phaseoloidin has been found to be a fish poison at a dilution of 125 mg/l of water (to be published).

Table 1. ^{13}C NMR spectral data of compounds **1a** (100 MHz, DMSO-d_6) and **4** (25.0 MHz, Me_2CO-d_6)

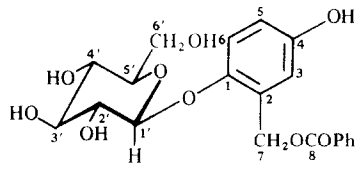
Carbon number	**1a**	**4**
1′	103.06	102.81
2′	73.52	73.71
3′	76.96	76.68
4′	69.96	70.11
5′	76.64	76.44
6′	61.01	61.38
1	152.17	152.37
2	126.04	127.52
3	117.15	116.41
4	148.61	148.87
5	117.70	118.70
6	114.15	116.02
7	35.05	134.15*
8	172.92	167.78

*Presumably an error in the original reference [13].

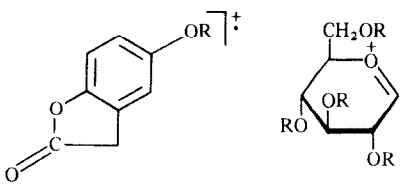
EXPERIMENTAL

Mps: uncorr. CC: silica gel (60–120 mesh; Glaxo, India); deactivated silica gel refers to above silica gel treated with 9% H_2O. TLC: silica gel G (Merck, India). The seeds of the plant were collected from the local market and identified by the Keeper, National Botanic Garden, Shibpur, Howrah 711 103, West Bengal by comparison with a herbarium specimen.

Extraction and isolation of phaseoloidin (**1a**). Air-dried powdered defatted seed kernel (3.5 kg) was extracted with hot aq. EtOH (70%). The glycosides were pptd by addition of a large vol. of Et_2O to the conc. extract. The Et_2O-insoluble gummy ppt. was dissolved in aq. MeOH and adsorbed on silica gel (300 g) for CC. It was packed on a column of deactivated silica gel (800 g) and eluted with solvents of increasing polarity, EtOAc–MeOH (17:3) eluate gave a crystalline compound, homogeneous on TLC (EtOAc–MeOH–HOAc, 80:13:7; spray soln: Liebermann–Burchard reagent). It crystallized from EtOAc–MeOH to give phaseoloidin (**1a**), mp 207–209°, $[\alpha]_D^{25}$ $-41.13°$ (H_2O; *c* 1.24), yield 4.3 gm. Found: C, 50.72; H, 5.78%. $C_{14}H_{18}O_9$ requires: C, 50.91; H, 5.46%. λ_{max}^{EtOH} nm (log ε): 228 (3.41) and 285 (3.35); $\lambda_{max}^{EtOH-KOH}$ nm: 237 and 302; ν_{max}^{KBr} cm^{-1} : 3550–3100 (*br*, OH, sugar moiety), 1700 (*s*, CO_2H), 1230 and 1045 (*s*, aromatic ether), 1650, 1620 and 1490 (aromatic); FDMS *m/z* 331 $[M+H]^+$; EIMS *m/z* (rel. int.): 312 (2) $[M-H_2O]^+$, 276 (1) $[M-3\times H_2O]^+$, 168 (23) [aglycone]$^+$, 150 (100) $[168-H_2O]^+$, 122 (54) $[150-CO]^+$, 94 (5) $[122-CO]^+$; ^1H NMR (90 MHz, DMSO-d_6): δ 3.28–3.36 (5H, *m*, H-2′,3′,4′,6′), 3.36–3.85 (3H, *m*, H-5′, benzylic CH_2), 4.58 (1H, *d*, $J=7$ Hz, H-1′), 5.17 (1H, *br*, disappeared on D_2O shake, phenolic OH), 6.66 (1H, *d*, $J=9$ Hz), 6.71 (1H, *d*, $J=2.5$ Hz) and 7.06. (1H, *dd*, $J=9$ and 2.5 Hz) (1,2,4-trisubstituted benzene moiety).



4



a R = H **b** R = Ac
d R = Me **c** R = H

Phaseoloidin pentaacetate (**1b**). Phaseoloidin (100 mg) on treatment with pyridine (0.5 ml) and Ac_2O (1 ml), followed by usual work-up and purification by repeated CC over silica gel yielded **1b** as a homogeneous (TLC) material, mp 65°; EIMS *m/z* (rel. int.): 480 (5) $[M-HOAc]^+$, 331 (11) (*b*), 271 (5) [*b*-HOAc]$^+$, 192 (26) [aglycone part $-H_2O]^+$, 169 (84) $[271-Ac_2O]^+$, 150 (100) $[192-CH_2CO]^+$, 122 (83) $[150-CO]^+$, 109 (85) $[169-HOAc]^+$; ^1H NMR (90 MHz, $CDCl_3$): δ 2.08 (12H, *s*, 4 × OAc), 2.27 (3H, *s*, phenolic OAc), 3.65 (2H, *m*, $ArCH_2CO_2H$), 3.87 (1H, *br*, H-5′), 4.23 (2H, *m*, H_2-6′), 4.82 (1H, *br*, H-1′), 5.03–5.50 (3H, *m*, H-2′, 3′, 4′), 6.93–7.17 (3H, *m*, aromatic).

Treatment of **1a** *with* CH_2N_2. Compound **1a** (125 mg) in MeOH on treatment with ethereal CH_2N_2 furnished a product purified by prep TLC (EtOAc–EtOH, 4:1) to yield **1c** as an amorphous homogeneous (TLC) compound, 60 mg; λ_{max}^{EtOH} nm: 223 and 285, no shift in alkali; EIMS *m/z* (rel. int.): 196 (53) [aglycone part]$^+$, 164 (14) [*d*; $196-MeOH]^+$, 163 (100) (*c*), 137 (30) $[196-CO_2Me]^+$, 136 (92) $[164-CO$ and/or $196-HCO_2Me]^+$.

Acetylation of **1c**. Compound **1c** (50 mg) on treatment with pyridine (1 ml) and Ac_2O (2 ml), followed by evapn to dryness and purification by CC over silica gel yielded in the $CHCl_3$–MeOH (99:1) eluate the homogeneous (TLC) tetraacetate (**1d**), mp 131–133°; λ_{max}^{EtOH} nm: 223 and 285; ν_{max}^{KBr} cm^{-1}: 1750, 1738 (CO_2Me, OAc), 1590, 1505, 1500 (aromatic), 1220, 1210, 1040

(OAc, aromatic ether); FDMS *m/z* 527 $[M+H]^+$; EIMS *m/z* (rel. int.): 526 (1) $[M]^+$, 467 (1) $[M-CO_2Me]^+$, 453 (1) $[M-CH_2CO_2Me]^+$, 331 (2) (*b*), 271 (1), 229 (2), 196 (5), 169 (60), 164 (10) (*d*), 136 (9), 109 (45), 43 (100); ^1H NMR (200 MHz, $CDCl_3$): δ 2.03, 2.04, 2.08, 2.09, (3H, *s*, each, 4 × OAc), 3.56 and 3.72 (1H, *d*, each, $J = 16$ Hz, H_2-7), 3.74 and 3.81 (3H, *s*, each, $-CO_2Me$ and Ar–OMe), 3.86 (1H, *br*, H-5'), 4.16 (1H, *dd*, $J = 12$ and 2.6 Hz, H-6'), 4.28 (1H, *dd*, $J = 12$ and 5.2 Hz, H-6'), 5.01 (1H, *d*, $J = 8$ Hz, H-1'), 5.14–5.38 (3H, *br*, H-2', 3', 4'), 6.77 (1H, *br d*, $J = 9$ Hz, H-6), 6.78 (1H, *br s*, H-3), 7.03 (1H, *br d*, $J = 9$ Hz, H-5).

Acid hydrolysis of **1a**. Phaseoloidin (100 mg) was hydrolysed by refluxing with aq. MeOH (50%)–conc HCl (22.5 ml: 2.5 ml) on steam-bath for 2 hr.

An aliquot of the acid hydrolysate was neutralized with Ag_2CO_3, the ppt. filtered off, and after usual work-up glucose was identified in the filtrate by descending PC (*n*-BuOH – HOAc $-H_2O$, 4:1:1; spraying reagent: aniline hydrogen phthalate).

The remaining acid hydrolysate was evapd to dryness and the residue purified by CC over deactivated silica gel, when $CHCl_3$–MeOH (49:1) eluate furnished the methyl ester **2b**, mp 118–121° ($CHCl_3$), 32.5 mg, EIMS *m/z* (rel. int.): 182 (15) $[M]^+$, 150 (65) (*a*), 149 (71), 123 (35) $[M-CO_2Me]^+$, 122 (100) [*a* $-CO]^+$, 94 (70) (*a* − 2 × CO); ^1H NMR (100 MHz, DMSO-d_6): δ 3.42 (2H, *s*, $ArCH_2CO_2Me$), 3.54 (3H, *s*, CO_2Me), 6.32 − 6.80 (3H, *m*, aromatic), 8.57 and 8.67 (1H, *s*, each disappeared on D_2O shake, 2 × phenolic OH).

Enzymatic hydrolysis of **1a**. Phaseoloidin (20 mg) in 0.1 M acetate buffer (pH 5.0; 35 ml) and β-glucosidase (Sigma U.S.A.; EC 3.2.1.21) (10 mg) were shaken at 37° for 2.5 hr. An aliquot of it was subjected to PC, as before, and the sugar part was identified as glucose.

The residual reaction mixture was evapd to dryness *in vacuo* at room temp. and the residue obtained was purified by CC over deactivated silica gel. The $CHCl_3$–MeOH (97:3) eluate yielded a compound which was crystallized from EtOH–$CHCl_3$ to give **2a**, mp 150-152° (lit. [14] 152°). This in MeOH was treated with ethereal CH_2N_2 and the reaction mixture showed one major and two minor products on TLC. The major compound was isolated by CC over deactivated silica gel and crystallized from $CHCl_3$ to give **2b**, mp 119–121°.

Treatment of **2b** *with* CH_2N_2. A MeOH soln of **2b** on treatment with etheral CH_2N_2 yielded two products which were purified by prep. TLC (C_6H_6–EtOAc–MeOH, 47:47:6; spraying agent: Liebermann–Burchard reagent) and identified as **2c** and **2d** by EIMS *m/z* 196 $[M]^+$ and 210 $[M]^+$, respectively.

Preparation of methyl ester of homogentisic acid. Homogentisic acid (10 mg) was refluxed with 1 N HCl in 50% aq. MeOH (2.5 ml) for 2 hr. Removal of the solvents *in vacuo* yielded a residue which was passed through a short column of deactivated silica gel. Elution with $CHCl_3$–MeOH (49:1) gave a product (7.5 mg), mp 119–120°, which was found to be identical with **2b** in all respects.

Esterification of **2a**. Compound **2a** obtained by the enzymatic hydrolysis of **1a** was esterified by refluxing with MeOH–HCl under similar conditions as in the above experiment. Similar work-up yielded **2b** in comparable yield.

Synthesis of methyl 2,4-dimethoxy phenyl acetate (**3b**). 2,4-Dimethoxy benzaldehyde (1.6 g) was heated with hippuric acid (1.92 g), powdered fused NaOAc (0.85 g) and Ac_2O (2.85 ml) on an oil-bath. Follow-up of the reaction mixture according to the reported procedure afforded 2-phenyl-4-(2',4'-dimethoxy) benzylideneoxazol-5-one as yellow needles, mp 178–181°(C_6H_6), yield 1.95 g.

Hydrolysis of the azlactone (1.75 g) with 10% aq. NaOH (9.5 ml) and subsequent treatment with dil. H_2O_2 (30% H_2O_2–H_2O, 1:1, 1.4 ml), followed by usual work-up yielded a mixture (1.5 g) of benzoic acid and 2,4-dimethoxy phenyl acetic acid.

Esterification of the above acid mixture with MeOH–H_2SO_4 (10 ml: 0.15 ml) and subsequent purification of the crude ester by CC over deactivated silica gel yielded in the *n*-hexane–C_6H_6 (1:1) eluate pure **3**, mp 53–54°, yield 1.2 gm; λ_{max}^{EtOH} nm: 228, 276 and 282; EIMS *m/z* (rel. int.): 210 (48) $[M]^+$, 179 (3), 151 (100), 121 (60), 91 (18); ^1H NMR (100 MHz, $CDCl_3$): δ 3.58 (2H, *s*, $ArCH_2CO_2Me$), 3.79 and 3.80 (3H, *s* each, 2 × ArOMe), 6.47 (1H, *dd*, $J = 7$ and 2.5 Hz), 6.50 (1H, *d*, $J = 2.5$ Hz), 7.11 (1H, *d*, $J = 7$Hz) (1,2,4-tri-substituted benzene moiety).

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