Phytochemistry, Vol. 27, No. 7, pp. 2313–2315, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

ISOLATION AND STRUCTURES OF ELLAGIC ACID DERIVATIVES FROM *EUPHORBIA ACAULIS*

R. S. BINDRA, N. K. SATTI and O. P. SURI*

Regional Research Laboratory (CSIR), Jammu 180001, India

(*Revised received* 25 *November* 1987)

Key Word Index—*Euphorbia acaulis*; Euphorbiaceae; 3,3′-di-*O*-methyl ellagic acid 4′-rutinoside; 3,4,3′-tri-*O*-methyl ellagic acid 4′-rutinoside.

Abstract—A new ellagic acid glycoside, 3,3′-di-*O*-methyl ellagic acid 4′-rutinoside, along with 3,4,3′-tri-*O*-methyl ellagic acid 4′-rutinoside and 3,4,3′-tri-*O*-methyl ellagic acid have been isolated from the rhizomes of *Euphorbia acaulis*. The structures of these compounds have been established on the basis of spectral (^{1}H NMR, ^{13}C NMR, MS, IR, UV) and chemical evidence.

INTRODUCTION

Euphorbia acaulis Roxb. is used by tribes of central India for curing various inflammatory disorders. The rhizomes of *E. acaulis* (which is called 'Banmuli' in the local language) in the form of a paste in mustard oil is recommended by the tribal medicine men for the treatment of gout and rheumatic disorders by external application. The anti-inflammatory activity of *Euphorbia acaulis* rhizome extract was established by a group of workers in our institution [1]. In view of its therapeutic properties, the rhizome of *E. acaulis* has been subject to chemical studies and in previous communications [2, 3] we reported the high resolution NMR and X-ray data of caudicifolin and a new entatis-16-ene diterpene, 3-oxoatisane-16α,17-diol. The present paper deals with the isolation of a new ellagic acid glycoside and two other compounds isolated for the first time from this species.

RESULTS AND DISCUSSION

Compounds **1**–**3** were isolated from the 50% aq. methanol extract of the rhizomes of *E. acaulis*. Compounds **1** and **3** gave a yellow colour with alkali and a dark bluish green precipitate with ferric chloride [4] indicative of the presence of a phenolic hydroxyl group. All the compounds gave a positive Greissmeyers reaction [5] indicative of ellagic acid derivatives.

The molecular formula of compound **1**, $C_{28}H_{30}O_{17}$, was obtained through exact mass measurement of the molecular ion $[M+Na]^+$ observed in the FAB mass spectrum and elemental analysis. The IR spectrum showed peaks at 3450 (*br* OH), 1740, 1700 (lactone carbonyl), 2940 and 1140 (–OMe), 1575, 1562, 1485 (aromatic) and 860 cm^{-1} (glycoside). Its UV spectrum (MeOH), showed absorptions at 249, 355 (sh) and 370 nm consistent with an ellagic acid derivative. The ^{1}H NMR spectrum of compound **1** showed two singlets of three protons each at δ3.77 and 3.87 for two methoxyl groups. Two singlets for one proton each at δ7.53 and 8.00 accounted for two aromatic protons and a doublet for 3H at 1.14 (J = 6 Hz) revealed the presence of one methyl group. The ^{13}C NMR spectrum of compound **1** revealed the presence of 28 carbon atoms in the molecule. The resonance frequencies of these atoms are summarized in Table 1.

Compound **1** on acid hydrolysis afforded an aglycone (**4**) and a mixture of two sugars identified by paper chromatography as D-glucose and L-rhamnose. The aglycone, $C_{16}H_{10}O_8$ (MS and elemental analysis), was determined to be 3,3′-di-*O*-methyl ellagic acid from its spectral data and by derivatization.

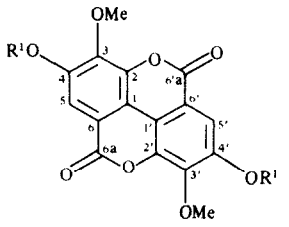
The glycoside **1** on permethylation with methyl iodide in the presence of sodium hydride and DMSO followed by hydrolysis gave 2,3,4-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-L-rhamnose along with compound **5**. The linkage of sugars was established by direct comparison by co-TLC, PC, GLC and ^{1}H NMR of the methylated sugars with those obtained on hydrolysis of permethylated

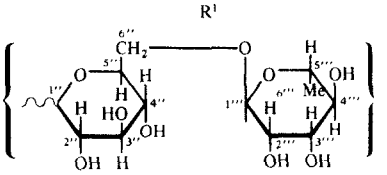
Table 1. ^{13}C NMR data of compounds **1** and **2** in DMSO-d_6

C	1	2	C	1	2
1	113.0 *s*	112.5 *s*	2″	74.5	73.8
2	143.0 *s*	142.7 *s*	3″	77.0	76.6
3	141.5 *s*	141.6 *s*	4″	70.5	70.5
4	154.0 *s*	154.7 *s*	5″	77.5	77.2
5	112.0 *d*	107.9 *d*	6″	67.0	66.7
6	113.8 *s*	112.6 *s*	1‴	101.5	100.6
6a	160.0 *s*	158.7 *s*	2‴	71.0	70.8
1′	113.0 *s*	112.5 *s*	3‴	71.5	71.3
2′	143.5 *s*	142.7 *s*	4‴	73.0	72.5
3′	142.2 *s*	141.6 *s*	5‴	69.0	68.7
4′	152.8 *s*	152.3 *s*	6‴	18.5	18.0
5′	115.2 *d*	113.5 *d*	3-OMe	62.0 *q*	61.9 *q*
6′	113.8 *s*	112.6 *s*	3′-OMe	62.5 *q*	62.2 *q*
6′a	160.0 *s*	158.5 *s*	4-OMe	—	57.5 *q*
1″	102.8	102.2	—	—	—

The assignments have been made by a combination of proton noise decoupled and SFORD spectra and by comparison with reported data.

*Author to whom correspondence should be addressed: P.G. Dept. of Chemistry, University of Jammu, Jammu 180001, India.





	R	R¹
1	H	
2	Me	
3 and **5**	Me	H
4	H	H
6	Ac	Ac
7 and **8**	Me	Ac
9	Me	Me

rutin. This confirmed the (6→1) intersugar linkage between D-glucose and L-rhamnose. Sodium periodate oxidation of compound **1** liberated two mol of formic acid with the consumption of four mol of periodate suggesting the presence of pyranosyl rings in both sugars.

Enzymatic hydrolysis (with emulsin) of compound **1** afforded compound **4** and the sugar rutinose, confirming the β-linkage between glucose with the aglycone. This led to the formulation of the glycoside **1** as 3,3′-di-*O*-methyl ellagic acid 4′-rutinoside.

The molecular formula of compound **2**, $C_{29}H_{32}O_{17}$, was obtained through exact mass measurement of the molecular ion $[M+Na]^+$ observed in the FAB mass spectrum and elemental analysis. The ^1H NMR, IR and UV data of **2** are presented in the Experimental while the ^{13}C NMR data along with probable assignments is presented in Table 1.

Compound **2** on acid hydrolysis afforded an aglycone (**5**) and a mixture of two sugars identified by PC as D-glucose and L-rhamnose. Compound **5** was identified as 3,4,3′-tri-*O*-methyl ellagic acid on the basis of physical constants and spectral data.

The pattern of linkage of sugars moiety in compound **2** was observed to be the same as in compound **1** and rutin by co-TLC, ^1H NMR and GLC of methylated sugars obtained by the hydrolysis of the permethylated glycosides. The NMR (^1H and ^{13}C) spectral data indicated that the sugar moieties in compounds **1** and **2** were same.

The data of compound **2** showed its close analogy with **1** while the molecular formulae indicated that instead of hydroxyl group at position 4 in compound **1**, **2** had a methoxyl function. These observations led to the formulation of **2** as 3,4,3′-tri-*O*-methyl ellagic acid 4′-rutinoside. Compound **2** has already been reported in the literature [6] as a constituent of *Erisma calcaratum* but its structure determination was not foolproof. Thus disaccharide glycoside yielding rhamnose and glucose as sugar moieties on hydrolysis could be neohesperidoside (2→1) or rhamnoglucoside (4→1) or rutinoside (6→1). In the present studies intersugar linkage has been determined in a systematic manner.

The molecular formula of compound **3**, $C_{17}H_{12}O_8$ was obtained by elemental analysis and EIMS ($[M]^+ = m/z$ 344). Compound **3** and its acetate derivative have spectral data exactly identical to those of compound **5** and its acetate. These compounds were also identical in respect of co-TLC, mp, and mmp. From the above data compound **3** was identified as 3,4,3′-tri-*O*-methyl ellagic acid.

EXPERIMENTAL

General. Mps: uncorr. The purity of the samples was checked on TLC on silica gel G. Paper chromatograms for sugars were run in the solvent system *n*-BuOH–HOAc–H_2O (4:1:5).

Plant material. Rhizomes of *Euphorbia acaulis* Roxb. were collected in the vicinity of Dudhwa National Park, Madhya Pradesh (India) and adjoining the territory of Nepal in the State of Uttar Pradesh between 27° 41′ and 28° 42′ N and 80° 20′ and 81° 19′ E. A herbarium specimen has been deposited at the National Botanical Research Institute, Lucknow, India.

Extraction, fractionation and isolation. Rhizomes of *E. acaulis* were extracted with 50% aq. MeOH. The residue left on the removal of the solvent was charged over a column of silica gel (60–120 mesh) and eluted with 9:1, 17:3 and 99:1 $CHCl_3$–MeOH to afford compounds **1**–**3** respectively in a fairly high state of purity. The final purification was done by crystallization.

Compound **1**. Light yellow crystals (hot MeOH), mp 226–227°; FABMS *m/z* 661 $[M+Na]^+$; Found: C, 52.52; H, 4.64%. Calc. for $C_{28}H_{30}O_{17}$, C, 52.66; H, 4.70%; UV λ_{max}^{MeOH} nm: 249, 355 (sh) and 370; IR ν_{max}^{KBr} cm^{-1}: 3450, 1740, 1700, 2940, 1140, 1575, 1562, 1485, 860; ^1H NMR (60 MHz, pyridine-d_5): δ1.14 (3H, *d*, *J* = 6 Hz, –Me), 3.77 and 3.87 (6H, 2*s*, 2 × –OMe), 7.53 and 8.00 (1H each 2*s*, Ar–H); ^{13}C NMR (22.49 MHz, DMSO-d_6): Table 1.

Acetylation of compound **1**. Acetylation of compound **1** with Ac_2O–C_5H_5N (100°, 6 hr) gave light yellow crystals, recrystallized from hot MeOH, mp 138–139°; ^1H NMR (60 MHz, pyridine-d_5): δ1.10 (3H, *d*, –Me), 1.76 (3H, *s*, 2‴ –OAc), 1.83 (3H, *s*, 3‴ –OAc), 1.91 (6H, *s*, 2″ –OAc and 3″ –OAc), 1.93 (3H, *s*, 4‴ –OAc), 2.00 (3H, *s*, 4″ –OAc), 2.13 (3H, *s*, OAc), 4.97–6.00 (12 H, *m*, sugar protons), 7.93 and 8.20 (1H each, 2 *s*, Ar –H).

Hydrolysis of compound **1**. Compound **1** (100 mg) in 100 ml MeOH was refluxed with 7% methanolic H_2SO_4 for 6 hr. The reaction mixture on cooling afforded light yellow crystals of aglycone **4** (recrystallized from MeOH) and a mixture of sugars. Compound **4**, mp 334–336°, MS *m/z*: 330, Found: C, 58.04; H, 3.05%. Calc. for $C_{16}H_{10}O_8$, C, 58.18; H, 3.03%. IR ν_{max}^{KBr} cm^{-1}: 3200, 1725, 1610; ^1H NMR (60 MHz, pyridine-d_5): δ4.23 (6H, *s*, 2 × –OMe), 8.01 (2H, *s*, Ar–H). The sugars after neutralization by passage through an ion exchange resin (OH) were identified as D-glucose and L-rhamnose on PC with authentic samples.

Acetylation of compound **4**. Compound **4** on heating with Ac_2O–pyridine (100°, 4 hr) afforded compound **6**, 3,3′-di-*O*-methyl ellagic acid diacetate, recrystallized from MeOH, mp, 300–303°; MS *m/z*: 414 $[M]^+$; IR ν_{max}^{KBr} cm^{-1}: 1760, 1605; ^1H NMR (60 MHz, $CDCl_3$): δ2.55 (6H, *s*, 2 × –OAc), 4.42 (6H, *s*, 2 × –OMe), 8.18 (2H, *s*, Ar–H).

Permethylation and hydrolysis of **1**. Compound **1** (100 mg) was dissolved in DMSO (10 ml) (Hakomori method) and mixed with a soln of NaH (450 mg) in DMSO (10 ml). The mixture was stirred for 1 hr under N_2, then MeI (3 ml) was added and stirring continued for 4 hr. The reaction mixture was poured in ice cold H_2O (300 ml) and extracted with EtOAc. The EtOAc extract was washed with H_2O (3 × 100 ml) and then concd *in vacuo*. The residue was taken up in 7% methanolic H_2SO_4 (100 ml) and refluxed for 6 hr on a steam bath. The reaction mixture was passed through an ion exchange resin (OH) and then chromato-

graphed on silica gel to give compound **5** along with 2,3,4-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose.

Compound **2**. Colourless crystals (aq. MeOH): mp 257°, FAB MS *m/z* 675 $[M+Na]^+$ Found: C, 53.20; H, 4.76%. Calc. for $C_{29}H_{32}O_{17}$ C, 53.37; H, 4.90%. UV λ_{max}^{MeOH} nm: 249, 355 (sh), 370: IR ν_{max}^{KBr} cm^{-1}: 3460–3410, 1740, 1720, 2900, 1125, 1590, 1565, 1485, 825; ^1H NMR (60 MHz, DMSO-d_6): δ1.01 (3H, *d*, –Me), 4.00, 4.01 and 4.13 (9H, 3*s*, 3 × –OMe), 4.87 (sugar protons, *d*, $J = 2$ Hz), 5.18 (sugar protons, *d*, $J = 7$ Hz), 7.46 and 7.80 (1H each, 2*s*, Ar–H); ^{13}C NMR (22.49 MHz, DMSO-d_6): Table 1.

Acetylation of compound **2**. Acetylation of compound **2** with Ac_2O–pyridine at room temp. overnight afforded a yellow crystalline solid, recrystallized from petrol–EtOAc, mp 149–150°: ^1H NMR (60 MHz, $CDCl_3$): δ1.15 (3H, *d*, $J = 6$ Hz, –Me), 1.93 (3H, *s*, 2‴–OAc), 2.0 (3H, *s*, 3‴–OAc), 2.06 (6H, *s*, 2″–OAc and 3″–OAc), 2.07 (3H, *s*, 4‴–OAc), 2.13 (3H, *s*, 4″–OAc), 4.05 (3H, *s*, OMe), 4.15 (6H, *s*, 2 × –OMe), 4.95–5.97 (12 H, *m*, sugar protons), 7.91 and 8.19 (1H each, 2*s*, Ar–H).

Hydrolysis of compound **2**. Compound **2** (200 mg) in 50% aq. MeOH (100 ml) was refluxed with 7% aq. H_2SO_4 for 6 hr. On cooling aglycone **5** was separated out from the reaction mixture, recrystallized from hot MeOH, light yellow crystals, mp 288–90°, MS *m/z* 344. Found: C, 58.05 H, 3.52%. UV λ_{max}^{MeOH} nm: 249, 355 (sh), 370: IR ν_{max}^{KBr} cm^{-1}: 3453, 1743, 1720 1580, 1565, 1485, ^1H NMR (60 MHz, DMSO-d_6): δ3.99, 4.04 and 4.06 (9H, 3*s*, 3 × –OMe) 7.48 and 7.54 (1H each, 2*s*, Ar–H). The sugars left in the mother liquor, after neutralization by passage through an ion exchange resin (OH), were identified as D-glucose and L-rhamnose by PC with authentic samples.

Acetylation of compound **5**. Acetylation of **5** with Ac_2O–pyridine (100°, 4 hr) afforded the monoacetate **7**, recrystallized from hot MeOH, mp 248–249°; ^1H NMR (60 MHz, $CDCl_3$): δ2.25 (3H, *s*,–OAc), 3.95, 4.13, 4.18 (9H, 3*s*, 3 × –OMe), 7.67 and 7.87 (1H each, 2*s*, Ar–H).

Permethylation and hydrolysis of **2**. Permethylation and hydrolysis of **2** (100 mg) was carried out by the Hakomori method to afford aglycone **5** along with 2,3,4-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose.

Compound **3**. Light yellow crystals (hot MeOH), mp 288–290°, MS, *m/z* 344. Found: C, 58.15; H, 3.42%. Calc. for $C_{17}H_{12}O_8$ C, 59.30; H, 3.48%. Compound **3** was identical to compound **5** in respect of spectral data, mp and co-TLC.

Acetylation of compound **3**. Acetylation of **3** with Ac_2O–pyridine (100°, 4 hr) afforded the monoacetate **8**, recrystallized from hot MeOH. Compound **8** was identical to compound **7** in respect of spectral data, mp, mmp and co-TLC.

Tetramethyl ether of compound **3**. On passing CH_2N_2 through a soln of **3** (MeOH) under ice cold conditions afforded tetra-*O*-methyl ellagic acid **9**, mp 353 (dec.) (Lit. mp 355 dec.), ^1H NMR (60 MHz, TFA): δ3.66, 3.96 (12 H, 2*s*, 4 × –OMe), 7.36 (2H, *s*, Ar–H).

Acknowledgement—The authors are grateful to Sh. Isher Dass for technical assistance and Instrumentation Division for spectral data. Our thanks are also due to Dr J. K. Maheshwari (National Botanical Research Institute, Lucknow) for providing plant material.

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