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6-*O*-MALONYL-β-METHYL-D-GLUCOPYRANOSIDE FROM ROOTS OF *RUMEX OBTUSIFOLIUS*

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Key Word Index—*Rumex obtusifolius*; Polygonaceae; roots; 6-*O*-malonyl-β-methyl-D-glucopyranoside; organic acids.

Abstract—From the methanol-soluble acidic fraction of *Rumex obtusifolius* 6-*O*-malonyl-β-methyl-D-glucopyranoside and ascorbalamic acid were isolated and identified. The identity of the former compound was confirmed by synthesis.

INTRODUCTION

Rumex obtusifolius (Japanese epithet, Ezo-no-gishigishi) is one of the most notorious weeds in fields and pastures in Japan. Roots of this plant remain undecomposed and reproduce new tissues after they are broken up in the soil by ploughing. The extermination of this weed is therefore very difficult. The constituents in *R. obtusifolius* roots have been surveyed in our laboratory and the isolation of ascorbalamic acid, γ-glutamyl peptides and furoylalanine (probably an artefact) from the acidic fraction was reported [1]. This paper reports the isolation of 6-*O*-malonyl-β-methyl-D-glucopyranoside, which is a new derivative of glucose, from the same fraction.

RESULTS AND DISCUSSION

The non-cationic fraction of the MeOH extract of *R. obtusifolius* roots contained some unusual organic acids besides those found ubiquitously. Two of them giving distinct spots with bromocresol green (BCG) on PC were isolated by the use of ion exchange and cellulose column chromatography and preparative PC and designated GOA-1 and -2 (R_f values, 0.43 and 0.21, respectively). GOA-2 was identified as ascorbalamic acid [1]. GOA-1 was obtained as a colourless, glassy material, which gave a positive reaction with BCG and a negative one to aniline phthalate. The ^{13}C NMR spectrum of GOA-1 in D_2O gave ten peaks: $\delta^{D_2O}_{TMS}$ (25 MHz): 42.0, 58.0, 64.8, 70.3, 73.9, 74.1, 76.4, 104.1, 170.0, 172.2. The chemical shifts of seven of them (58.0, 64.8, 70.3, 73.9, 74.1, 76.4, 104.1) were nearly the same as those of β-methyl-D-glucopyranoside, except two signals corresponding to C-5 and C-6 (76.4 and 64.8), which shifted to higher (3.1 ppm) and lower (2.7 ppm) field, respectively [2]. The signal of the highest field (42.0) corresponded to a methylene carbon between two carbonyl groups and two other peaks (170.0 and 172.2) were in the carboxyl region. The IR spectrum showed the presence of ester, free carboxyl and OH groups: ν^{KBr}_{max} cm^{-1}: 3400, 1740, 1400, 1320, 1280, 1200, 1160, 1080, 1040, 1020. The structure of the ester between the primary OH group of C-6 of β-methyl-D-glucopyranoside and malonic acid was deduced from these spectral data. The MW obtained by FDMS supported this structure. FDMS (*m/z*): 281 ($M^+ + 1$, 100%). Degradation reactions were carried out to confirm the structure. GOA-1 gave levulinic acid by hot acid treatment (6 N HCl, 120°, overnight), glucose under mild conditions (N HCl, 105°, 1 hr), β-methyl-D-glucopyranoside and dimethyl malonate with CH_2N_2, and malonic acid with 0.5 N $Ba(OH)_2$. From these degradation reactions it was confirmed that the structure of GOA-1 is 6-*O*-malonyl-β-methyl-D-glucopyranoside. Chromatographic behaviour, spectral data and optical rotation of GOA-1 were consistent with the values obtained for 6-*O*-malonyl-β-methyl-D-glucopyranoside synthesized from β-methyl-D-glucopyranoside and malonic acid.

A spot corresponding to 6-*O*-malonyl-D-glucose, which was also synthesized from D-glucose and malonic acid, was not detected on PC of the fraction containing GOA-1.

The study of the effects of 6-*O*-malonyl-β-methyl-D-glucopyranoside on the growth of bacteria, fungi and higher plant is in progress in relation to the resistance of roots of *R. obtusifolius* to decomposition in soil.

EXPERIMENTAL

PC and Avicel column chromatography were performed with *n*-BuOH–HOAc–H_2O(4:1:2). Organic acids were located by dipping the chromatogram into 0.1% BCG in Me_2CO. Amino acids and sugars were detected with 0.2% ninhydrin in Me_2CO and aniline–phthalic acid in *n*-BuOH satd with H_2O (1.66 g and 0.93 g in 100 ml), respectively. GC of organic acids was carried out by the method of ref. [3].

Isolation of 6-O-*malonyl-β-methyl-*D-*glucopyranoside* (*GOA*-1). Roots of *R. obtusifolius* (5.7 kg), collected in the farm of Hokkaido University on May 1979, were cut into small pieces and extracted with MeOH. The extract was concd and applied to a Dowex 1 × 4 (AcO^-, 1 l.) column. Neutral and basic substances were washed out with H_2O and the acidic fraction was eluted with 8 N HOAc (10 l.) and 2 N HCl (2 l.). Fractions (1 l.) were collected. The following compounds were identified by comparing R_f values on PC with authentic specimen and/or spectral analysis after isolation by prep. PC: monoMe malonate (probably an artefact; fr. 2,3; IR, ^1H NMR), malonic acid

(fraction (fr.) 1,2; IR, ^{1}H NMR), pyroglutamic acid (fr. 1,2; ^{1}H NMR), glutamic acid (fr. 1,2), aspartic acid (fr. 1,2), GOA-1 (fr. 1,2), GOA-2 (fr. 2), malic acid (fr. 3,4; ^{1}H NMR), citric acid (fr. 5 ~ 10; ^{1}H NMR), oxalic acid (fr. 6 ~ 12. IR). Fraction 2, which contained the largest amount of GOA-1 and -2, was concd and applied to an Amberlite IR-120 (H^+, 100 ml) column. Both GOA-1 (200 mg/kg) and -2 (20 mg/kg) were obtained as colourless, glassy substances after the effluent and H_2O wash from the column was treated by Avicel column chromatography and prep. PC. GOA-2 was identified as ascorbalamic acid by spectral analysis and degradation [1,4].

GOA-1. $[\alpha]_D^{16} - 26.0°$ (*c* 1.1, H_2O). ^{1}H NMR in D_2O (90 MHz); centre at 3.47 (*m*, 9 H, including *s* at 3.47), 4.34 (*m*, 3 H). See Results for ^{13}C NMR and IR spectra.

HCl *treatment*. GOA-1 (30 mg) was heated with 6 N HCl in a sealed tube (120°, 19 hr). The hydrolysate was concd and passed through Dowex 50 (H^+, 1 ml). Levulinic acid (9 mg) was obtained from the effluent and identified by comparison of the R_f value on PC with an authentic sample and by ^{1}H NMR and MS. GOA-1 (20 mg) was dissolved in N HCl and heated to 105° for 1 hr in a sealed tube. Glucose was detected on PC of the hydrolysate.

CH_2N_2 *treatment*. GOA-1 (40 mg) was dissolved in MeOH (1 ml) and treated with excess CH_2N_2–Et_2O. The reaction mixture was concd and β-Me-D-glucopyranoside (38 mg) crystallized from MeOH–$CHCl_3$. FDMS (*m/z*); 195 (M^+ + 1, 95.4%). IR and ^{1}H NMR spectra and optical rotation agreed with those of an authentic specimen. $[\alpha]_D^{16} - 33.8°$ (*c* 0.74, H_2O) (lit. −31°, *c* 8, H_2O [5]). The existence of diMe malonate in the mother liquor was demonstrated by GC and confirmed by MS. MS (*m/z*); 101 (M^+ − OMe, 100), 74 (M^+ − COOMe + H, 78.9).

Alkaline treatment. GOA-1 (46 mg) was dissolved in 0.5 N $Ba(OH)_2$ (1 ml) and kept overnight at room temp. The reaction mixture was acidified with 6 N HCl (pH 1) and extracted with EtOAc. The extract was concd and malonic acid (7 mg) was crystallized out from Et_2O–hexane. The IR spectrum was the same as that of authentic malonic acid.

Synthesis of 6-O-malonyl-β-methyl-D-glucopyranoside. Dicyclohexylcarbodiimide soln in dry dioxane (0.015 mol in 30 ml) was added dropwise to a suspension of β-Me-D-glucopyranoside (0.015 mol) and malonic acid (0.015 mol) in dry dioxane (30 ml) at room temp. with vigorous stirring. After stirring 2 days, H_2O was added to the reaction mixture and filtered. The filtrate was concd and applied to a Dowex 1x4(AcO^-, 35 ml) column, which was washed with H_2O and eluted with 2 N HOAc. Fractions of 50 ml were collected. 6-*O*-Malonyl-β-Me-D-glucopyranoside (1.7 g, yield; 40%) was obtained as a colourless, glassy product from fr.1 and 2. IR, ^{1}H NMR and ^{13}C NMR spectra, chromatographic behaviour and optical rotation of GOA-1 and the synthetic compound were identical. $[\alpha]_D^{16} - 22.9°$ (*c* 1.3, H_2O). FDMS (*m/z*); 281 (M^+ + 1, 100%).

Synthesis of 6-O-malonyl-D-glucose. The compound was prepared from D-glucose and malonic acid by the same procedure as that employed for the prepn of 6-*O*-malonyl-β-Me-D-glucopyranoside. $[\alpha]_D^{16} + 27.0°$ (*c* 0.74, H_2O). FDMS (*m/z*); 305 (M^+ + K, 18.3%), 289 (M^+ + Na, 100), 267 (M^+ + 1, 47).

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REFERENCES

1. Kasai, T., Okuda, M. and Sakamura, S. (1980) *Agric. Biol. Chem.* **44**, 2723.
2. Dorman, D. E. and Roberts, J. D. (1970) *J. Am. Chem. Soc.* **92**, 1355.
3. Yamashita, I., Tamura, T., Yoshikawa, S. and Takanami, S. (1974) *Nippon Nogei Kagaku Kaishi* **48**, 165.
4. Couchman, R., Eagles, J., Hegarty, M. P., Laird, W. M., Self, R. and Synge, R. L. M. (1973) *Phytochemistry* **12**, 707.
5. Dawson, R. M. C., Elliott, D. C., Elliott, W. H. and Jones, K. M. (eds.) (1969) *Data for Biochemical Research*, 2nd edn, p. 239. Clarendon Press, Oxford.