Pergamon

PII: S0031-9422(96)00276-2

Phytochemistry, Vol. 43, No. 1, pp. 141–143, 1996
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0031-9422/96 $15.00 + 0.00

2-ETHYL-3-METHYLMALEIMIDE *N*-β-D-GLUCOPYRANOSIDE FROM THE LEAVES OF MANGOSTEEN (*GARCINIA MANGOSTANA*)

Doris Krajewski, Gábor Tóth* and Peter Schreier†

Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany; *Technical and Analytical Research Group of the Hungarian Academy of Science, Institute for General and Analytical Chemistry, Technical University Budapest, 1111 Budapest, St. Gellért tér 4, Hungary

(*Received* 14 *March* 1996)

Key Word Index—*Garcinia mangostana*; Guttiferae; mangosteen leaves; 2-ethyl-3-methylmaleimide *N*-β-D-glucopyranoside; 3-ethyl-4-methyl-1*H*-pyrrole-2,5-dione; APCIMS; HMBC.

Abstract—From a methanolic extract of leaves of *Garcinia mangostana*, 2-ethyl-3-methylmaleimide *N*-β-D-glucopyranoside was isolated by adsorption chromatography on XAD resin followed by rotation locular countercurrent chromatography. After acetylation, separation was performed by column chromatography on silica gel and preparative HPLC on RP-18 phase. Identification was carried out by mass spectrometry (DCI, APCI) and nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR, ^{1}H ^{1}H-COSY, HMQC). Assignment of the quateranry carbon resonances was achieved by HMBC experiments. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In plants, many natural constituents, such as nucleic acid bases, nicotinic acid or cytokinin phytohormones and xenobiotics are known to form *N*-glycosides. The conjugation process proceeds enzymatically by *N*-glycosyltransferases with activated nucleotide sugars like PRPP or UDP-glucose [1–4]. This paper deals with the isolation and structural elucidation of a new *N*-glucosidically bound flavour compound from mangosteen (*Garcinia mangostana*) leaves. Previous interest in mangosteen has focused on biologically active xanthones [5] and fruit flavour constituents [6].

RESULTS AND DISCUSSION

From a mangosteen leaf extract obtained by XAD adsorption and subsequent methanol elution, compound **1** was separated by rotation locular countercurrent chromatography (RLCC) and, after acetylation, by subsequent LC on silica gel and also HPLC on RP-18 phase. The elemental composition of the isolated pure compound **1** was determined to be $C_{21}H_{27}NO_{11}$, 469 amu, by HR-DCI-mass spectrometry corresponding to a tetraacetylated monoglycoside. HPLC–tandem mass spectrometry under atmospheric pressure ionization (APCI) gave the adduct ions $[M+H]^+$, *m/z* 470, and $[M+NH_4]^+$, *m/z* 487. The CID spectrum of *m/z* 470 was dominated by two fragmentation pathways resulting from the successive loss of ketene, $CH_2{=}C{=}O$, *m/z* 42, or acetic acid from either the entire molecule or the anhydrosugar moiety, *m/z* 331 $[M-\text{aglycone}+H]^+$. The first gave raise to the fragments *m/z* 428, 410, 368, 350, 308, 290, 248, the second to *m/z* 271, 211, 169, 109, corresponding to a cleavage along the *N*-glycosidic bond [7, 8]. ^{1}H, ^{13}C, ^{1}H–^{1}H COSY and HMQC NMR experiments (Tables 1 and 2) clearly revealed four glucose linked acetyl groups and the signals attributable to a β-D-*N*-tetraacetylglucose owing to the highfield shifted anomeric carbon (δ 77.6) and lowfield shifted anomeric proton (δ 5.20, $J = 9.5$ Hz) [9].

Double-resonance experiment by decoupling of H-5′ (δ 3.78) confirmed H-4′ (δ 5.20, $J = 9.4$ Hz) to coincide with H-1′. The aglycone showed only signals of an ethyl (H_2-C5 δ 2.41 and C-5 δ 17.2; H_3-C6 δ 1.14 and C-6 δ 12.4) and methyl group (H_3-C7 δ 1.97 and C-7 δ 8.6). Upon irradiation of H_2-C5 and H_3-C7 resonances in the NOE difference experiment, enhancement of the signal intensity revealed them to be placed in cisoid relationship on a double bond. From these data, together with the UV (λ_{max} 220 nm) and IR spectroscopical data (ν 1750, 1710 cm^{-1}), the compound was considered to be the peracetylated *N*-β-D-glucopyranoside of 2-ethyl-3-methylmaleimide [10, 11]. The four quaternary carbons were finally detected by remarkably sensitive HMBC by long-range $^2J_{CH}$ and $^3J_{CH}$ H–C couplings. Respective cross-peaks (Table 1) led to the unequivocally assignments of the double-bond carbons C-2 (δ 143.0) and C-3 (δ 137.4) as well as the carbonyl carbons C-1 (δ 169.6) and C-4 (δ 170.1). The *N*-glucoside **1** was found to be chemically stable under acidic conditions owing to resonance

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†Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR data of compound **1** (600 MHz, δ in ppm, coupling constants in Hz, CDCl$_3$)

H	δ	**1**	Cross-peaks in HBMC (J = 5 Hz) $^2J_{CH}$	$^3J_{CH}$
5	2.41	2H, *q* (7.6)	C-2	C-1,3
6	1.14	3H, *t* (7.6)		C-2
7	1.97	3H, *s*	C-3	C-2,4
Glucose				
1′	5.20	1H, *d* (9.5)	C-2′	C-1,4
2′	5.92	1H, *t* (9.4)	C-1′,3′	CH$_3\underline{\text{C}}$O-2′
3′	5.27	1H, *t* (9.4)	C-2′,4′	CH$_3\underline{\text{C}}$O-3′
4′	5.20	1H, *t* (9.7)	C-3′,5′	CH$_3\underline{\text{C}}$O-4′
5′	3.78	1H, *ddd* (9.9; 4.4; 2.5)		
6a′	4.17	1H, *dd* (12.5; 2.5)	C-5′	CH$_3\underline{\text{C}}$O-6′
6b′	4.19	1H, *dd* (12.5; 4.6)	C-5′	CH$_3\underline{\text{C}}$O-6′
C$\underline{\text{H}}_3$CO-2′	1.93	3H, *s*	CH$_3\underline{\text{C}}$O-2′	
C$\underline{\text{H}}_3$CO-3′	2.02	3H, *s*	CH$_3\underline{\text{C}}$O-3′	
C$\underline{\text{H}}_3$CO-4′	2.04	3H, *s*	CH$_3\underline{\text{C}}$O-4′	
C$\underline{\text{H}}_3$CO-6′	2.08	3H, *s*	CH$_3\underline{\text{C}}$O-6′	

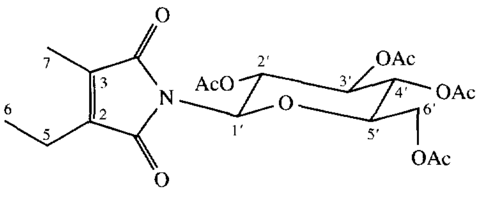
Table 2. ^{13}C NMR data of compound **1** (150 MHz, δ in ppm, CDCl$_3$)

C	δ	DEPT
1	169.6*	C
2	143.0*	C
3	137.4*	C
4	170.1*	C
5	17.2	CH$_2$
6	12.4	CH$_3$
7	8.6	CH$_3$
Glucose		
1′	77.6	CH
2′	68.1	CH
3′	73.7	CH
4′	67.9	CH
5′	74.5	CH
6′	61.8	CH$_2$
$\underline{\text{C}}$H$_3$CO-2′	20.4*	CH$_3$
$\underline{\text{C}}$H$_3$CO-3′	20.6*	CH$_3$
$\underline{\text{C}}$H$_3$CO-4′	20.6*	CH$_3$
$\underline{\text{C}}$H$_3$CO-6′	20.7*	CH$_3$
CH$_3\underline{\text{C}}$O-2′	168.9*	C
CH$_3\underline{\text{C}}$O-3′	170.1*	C
CH$_3\underline{\text{C}}$O-4′	169.3*	C
CH$_3\underline{\text{C}}$O-6′	170.6*	C

*Assigned by HMBC-experiment.

delocalization of the free electrons of the anomeric nitrogen atom [12, 13].

The aglycone 2-ethyl-3-methylmaleimide has been reported previously as flavour constituent in fresh plant tissue [14, 15], in wine [16] and in processed leaves such as tobacco [17, 18] and tea [19, 20]. It has also been detected in a model system after photodegradation of chlorophyll a, a good singlet oxygen sensitizer [22].



1

EXPERIMENTAL

General. TLC was carried out on SiO$_2$ 60 F$_{254}$ (Merck) using the less-polar RLCC layer as mobile phase (detection, 254 nm and vanillin/H$_2$SO$_4$). Flash chromatography was performed on silica gel 60 (activity grade II, 0.032–0.063 mm). RLCC was used in the ascending mode (CHCl$_3$–MeOH–H$_2$O, 7 : 13 : 8). For analytical HPLC an Eurospher 100 C-18 column (5 μm, 250 × 4 mm, Knauer) and diode array detection with scanning from 190 to 400 nm were used. Prep. HPLC was performed on Eurospher 100 C-18 (5 μm, 250 × 16 mm, Knauer) and UV detection at 220 nm. The following solvents were used: (1) analytical HPLC: H$_2$O–MeOH gradient with 1–99% MeOH in 45 min (1 ml min^{-1}), (2) prep. HPLC: H$_2$O–MeOH gradient with 40–100% MeOH in 30 min (5 ml min^{-1}). HRGC-MS analysis was carried out using a J&W DB-5 fused silica capillary column (30 m × 0.25 mm i.d., df = 0.25 μm). Split-injection (1 : 20) was employed. Temp. programme: 60–300° at 5° min^{-1}. The flow rate for the carrier gas was 2.5 ml min^{-1} He. The temp. of the ion source and all connection parts was 220° and of the injector 250°, electron energy 70 eV and cathodic current 0.7 mA. DCI spectra were carried out with NH$_4$ and CH$_4$ as reactant gases, electron energy was 70 eV. Source pressure was held at 0.4 mbar and temperature at 140°. HPLC–MS/MS under atomspheric pressure ionization (APCI): flow rate 200 μl, corona voltage 4 kV, temperature of the heated inlet capillary 180°, vaporizer 300°. Nitrogen served both as sheat (344.7 kPa) and auxiliary gas (10 ml min^{-1}). Positive ions were detected scanning from 100 to 600 u with a total scan duration of 1.0 sec and a dwell time of 2 msec. MS/MS experiments were performed at a collision pres. of 1.80 mTorr argon, mass range 50–500 u, collision offset C_{off} −10 eV, respectively. Electron multiplier voltage was set to 1200 V in scan mode and 1800 V for MS/MS experiments. All NMR spectra were taken in $CDCl_3$ and referenced to TMS. ^1H and ^{13}C NMR spectra were measured at 600/150 MHz. NOE difference and homonuclear double-resonance experiments were recorded at 400 MHz, HMBC ($J(CH)_{opt} = 5$ Hz) at 500 HMz.

Plant material. Mangosteen leaves (*G. mangostana* L.) were harvested in October 1993 in Mariquita, Colombia.

Extraction and isolation of compound **1**. A crude glycosidic extract (85 g from 8 kg of fresh leaves) was obtained by adsorption on XAD-2 (Serva) resin and elution with MeOH as described previously [22]. Further fractionation by RLCC yielded combined frs I–VI. RLCC fraction IV (2 g) was acetylated using standard Ac_2O-pyridine procedure at room temp. for 12 hr in the dark. Purification was carried out by flash chromatography under N_2 pressure with pentane, EtO_2 and $CHCl_2$ as solvents (pentane–EtO_2: 300 ml 100:0; 300 ml 20:80; 500 ml 0:100; 200 ml $CHCl_2$), frs of 20 ml being collected. The sepn was monitored by TLC and the pooled frs I–X were checked by analytical HPLC. The residue of fr. III (73 mg) was purified by prep. HPLC to give 3 mg of **1**.

Acid degradation study. An aliquot of deacetylated **1** was resolved in 1.5 N H_3PO_4 and heated for 1.5 hr at 100°. The aq. phase was extracted with Et_2O and the organic layer analysed by means of HRGC-MS.

2-Ethyl-3-methylmaleimide 2,3,4,6-*tetraacetyl* N-β-D-*glucopyranoside* (**1**). UV λ_{max}^{MeOH} nm: 220, 278 sh. IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 2960, 1750 (>C=O), 1710 (>C=O), 1350, 1220. HRDCI-MS (methane): 470.1655 $[M+H]^+$ ($C_{21}H_{28}NO_{11}$ requires 470.1648). DCI-MS (methane) *m/z* (rel. int.): 470 $[M+H]^+$ (100), 410 $[M+H-HOAc]^+$ (69), 350 $[410-HOAc]^+$ (13), 331 $[M-\text{aglycone}+H]^+$ (33), 271 $[331-HOAc]^+$ (5), 89 (100). DCI-MS (NH_3) *m/z* (rel. int.): 487 $[M+NH_4]^+$ (100). APCI HPLC–MS *m/z*: 487 $[M+18]^+$, 470 $[M+H]^+$, 410 $[470-HOAc]^+$, 331 $[M-\text{aglycone}+H]^+$. LC-MS/MS of *m/z* 470 (C_{off} − 10 eV): 470 $[M+H]^+$, 428 $[470-CH_2{=}C{=}O]^+$, 410 $[470-HOAc]^+$, 368 $[410-CH_2{=}C{=}O]^+$, 350 $[410-HOAc]^+$, 331 $[M-\text{aglycone}+H]^+$, 308 $[350-CH_2{=}C{=}O]^+$, 290 $[350-HOAc]^+$, 271 $[331-HOAc]^+$, 248 $[290-CH_2{=}C{=}O]^+$, 211 $[271-HOAc]^+$, 169 $[211-CH_2{=}C{=}O]^+$, 109 $[169-HOAc]^+$. HRGC-MS (EI) data: R_i (DB-5) 2616; *m/z* (rel. int. %): 43 (100), 57 (7), 81 (21), 98 (13), 112 (11), 115 (13), 139 (7), 140 (15), 141 (10), 168 (40), 169 (8), 234 (20), 247 (12), 276 (13), 289 (16), 307 (7).

Acknowledgements—This work was supported financially by the EU (AIR3-CT94-2193). G. T. thanks the Hungarian Research Foundation (OTKA Grant No. T 014978) and National Committee for Technological Development (OMFB Grant No. 107) for financial support. We are indebted to Prof. C. Duque, Universidad Nacional de Colombia, Bogota, Colombia, for providing the plant material. We gratefully acknowledge Dr Scheutzow's and E. Ruckdeschel's NMR analyses and Dr G. Lange's DCI mass spectra analyses (Institut für Organische Chemie). We thank E. Richling for the measurement of the APCI mass spectra.

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