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Note

A Rubrofusarin Gentiobioside Isomer from Roasted *Cassia tora*

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From the roasted seeds of *Cassia tora* L., a new naphthopyrone glycoside was isolated and characterized as 10-[(β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl)oxy]-5-hydroxy-8-methoxy-2-methyl-4H-naphtho[1,2-*b*]pyran-4-one(isorubrofusarin gentiobioside). Along with isorubrofusarin gentiobioside, alaternin and adenosine were isolated and identified.

Key words : roasted, *Cassia tora*, Leguminosae, naphthopyrone glycoside, isorubrofusarin gentiobioside

INTRODUCTION

The dried or roasted seed of *Cassia tora* L. (Leguminosae) is used as a Chinese herbal medicine to improve vision, and is also reputed for its medicinal value as an asperient, antiasthenic, and diuretic agent (Namba, 1980). We have previously reported that the methanoic extract of the seeds of *C. tora* possess a radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Choi, *et al.*, 1993). From this methanolic extract, 2-hydroxy emodin(alaternin), cassiaside and rubrofusarin gentiobioside were isolated as the active principles, together with the inactive components of chrysophanol, physcion, β-sitosterol, chryso-obtusin, emodin, aurantio-obtusin, cassitoroside, and chrysophanol triglucoside (Choi, *et al.*, 1994, 1995). We initiated a study on roasted Cassia seeds because the roasted seed of *C. tora* is frequently consumed as a tea preparation in Korea. In this paper, we report on the isolation and identification of a new naphthopyrone glycoside, isorubrofusarin gentiobioside, which is likely to be produced by this roasting process.

MATERIALS AND METHODS

A UV spectrum was taken using a Shimazu 202 UV spectrophotometer in MeOH, and an IR spectrum was taken on a JASCO IR-2 spectrometer in KBr disc. FAB-MS was obtained with a HR Tandem mass spectrometer using a direct inlet system with NBA being used as a matrix. ^{1}H- and ^{13}C-NMR spectra were taken with a Varian UNITY-300 spectrometer. The chemical shifts were referenced to a residual solvent peak (2.5 ppm in ^{1}H-NMR, 39.5 ppm in ^{13}C-NMR). The column chromatography was undertaken with a silica gel (Merck, 70~230 mesh). The TLC was carried out on a precoated Merck Kieselgel 60 F_{254} plate (0.25 mm), and spots were detected under a UV light using a 50% H_2SO_4 reagent.

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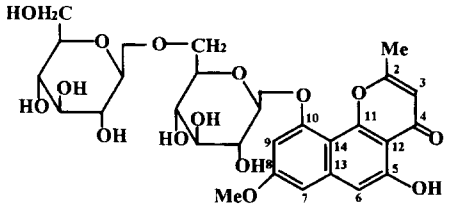
Plant materials

The seeds of *Cassia tora* were purchased from a commercial supplier in 1993, and authenticated by Prof. H. J. Chi. A voucher specimen has been deposited in the Herbarium of the Natural Products Research Institute, Seoul National University.

Isolation of compounds

Cassia seed (1.6 kg) roasted at 250°C for 10 min was grounded and extracted with MeOH and concd to give a dark residue, which was partitioned between *n*-hexane and H_2O. The H_2O-soluble material was further partitioned with dichloromethane, and then *n*-butanol to give a butanol-soluble fraction (18.4 g). This was subjected to CC on silica gel and eluted with mixtures of dichloromethane and methanol of increasing polarity. The eluates were collected in 250 ml portions, monitored by TLC, and finally combined into eighteen fractions. A fraction 14 (1.8 g) was rechromatographed on a silica gel column, with dichloromethane-MeOH (10:1) to give compound 1 (180 mg). Fractions 2 and 3 were combined and rechromatographed on a silica gel column, with dichloromethane-MeOH (10:1) to give alaternin, mp 296~298°C (Choi, *et al.*, 1994). Nucleoside adenosine was isolated from fraction 13, mp 225~225°C (Choi, *et al.*, 1992). The un-



Structure of compound 1

roasted Cassia seed was similarly ground, extracted and partitioned. The TLCs of the fractions from the roasted and unroasted seed were compared with each other, and the spots of **1** was detected only in the TLC (R_f=0.41, solvent; CH_2Cl_2:MeOH:H_2O=65:35:10, lower layer) of the *n*-BuOH soluble fraction from the roasted seed.

Compound 1 (isorubrofusarin gentiobioside): A pale yellow amorphous powder; UV λ_{max}^{MeOH} nm (logε): 240 (4.66), 279 (4.42), 370 (3.64); IR ν (cm^{-1}); 3350, 1655, 1615, 1565, 1460, 1370, 1310, 1200, 1050; Positive ion FAB-MS [matrix, NBA] *m/z*; 597 $[MH]^+$, 435 $[M\text{-}Glu+H]^+$, 272 $[M\text{-}2Glu]^+$; MS (EI) *m/z* (rel. int.); 272 $[M^+\text{-}Gen, 100]$; ^{1}H- and ^{13}C-NMR: Table I.

RESULTS AND DISCUSSION

The column chromatography of the *n*-butanol-soluble part of the methanol extract of the roasted seeds yielded a pale yellow powder (**1**), which produced a dark blue spot on UV illumination, and a positive Molisch test. The IR spectrum of **1** showed absorption bands due to γ-pyrone, α,β-unsaturated carbonyl, and an aromatic ring at 1,655, 1,615, and 1,560 cm^{-1}, respectively. Strong absorption bands at 3,350 and 1,100~1,000 cm^{-1} were observed which would indicate its glycosidic nature. The UV spectrum of compound 1 exhibited typical absorption maxima for non-linear naphtho-γ-pyrone at 240, 279 and 370 nm (Gorst-Allman *et al.*, 1980). The quasi-molecula ion peak at *m/z* 597$[MH^+]$ in the positive-ion FAB-MS was consistent with the molecular formula $C_{27}H_{32}O_{15}$. A successive loss of two glucose units from the parent molecular ion to give the aglycone ion at *m/z* 272 showed the presence of a diglucoside unit. The ^{1}H-NMR spectrum of **1** in DMSO-d_6 (Table I) exhibited the presence of a methyl (δ 2.53), a methoxyl (δ 3.87), four aromatic protons ascribable to an isolated (δ 6.93) and a pair of *meta*-coupled ones (δ 6.90, 6.80, J=2.4 Hz) as well as an exchangeable hydroxy proton at δ 12.92. It also showed proton signals due to the sugar moieties between δ 3.10~5.14 including two anomeric proton signals (δ 4.20, 5.14). As can be seen in Table I, the sugars would appear to be β-D-gentiobioside according to ^{13}C-NMR data. A detailed analysis of the ^{1}H- and ^{13}C-NMR spectra (Table I), aided by HMQC (Bax and Subramanian, 1986) and HMBC (Bax and Summers, 1986) experiments, enabled the establishment of the structure of **1**. The ^{13}C-signals of the protonated carbones in **1** were readily assigned by careful analysis of the HMQC spectrum and by comparisons with the ^{13}C-NMR data of the related linear naphthopyrone glycosides, and non-linear flavasperone and isonigerone (Choi *et al.*, 1994, Gorst-Allman *et al.*, 1980, Messana *et al.*, 1991). The UV spectrum of the angularly fused flavasperone was fundamentally different from that of the linear compound rubrofusarin, and as the UV spectrum of **1** is partically superimposable on that of flavasperone, a non-linear structure is indicated for **1** (Gorst-Allman *et al.*, 1980). The ^{1}H-NMR data (Table I) show that the resonance position of the C-5 hydroxy-proton is also diagnostic of the non-linear isomer. For the non-linear compounds, the hydroxy-proton is weakly hydrogen-bonded to the carbonyl group and resonates around δ 13, whereas in the linear compounds the hydrogen bonding is more stronger, with the hydroxy-proton appearing around δ 15. A comparison of the ^{13}C resonances of C-5 in rubrofusarin gentiobioside (δ 183.7) (Choi *et al.*, 1994)

Table I. ^{1}H- and ^{13}C-NMR data of **1** in DMSO-d_6 (coupling constants in Hz in parentheses)

Position	**1**[a]	
	δ_H	δ_C
2	------	168.35
3	6.47s	109.62
4	------	182.30
5	------	155.60
6	6.93s	104.91
7	6.90d (2.4)	99.53
8	------	161.13
9	6.80d (2.4)	100.05
10	------	156.06
11	------	155.15
12	------	108.08
13	------	140.25
14	------	104.19
1'	5.14d (7.2)	100.31
2'	------	73.65*
3'	------	76.91
4'	------	70.09
5'	------	75.42
6'	4.20d (7.8)	68.70
1"	------	103.55
2"	------	73.53*
3"	------	76.78
4"	------	69.54
5"	------	76.66
6"	3.87s	61.05
8-OCH_3	2.53s	55.48
2-CH_3	12.92s	19.83
5-OH		------

[a]Assignments are based on the analysis of DEPT, HMQC, and HMBC data.
*Assignments may be reversed.

and **1** (δ 182.30) substantiates the relatively weaker hydrogen bonding in **1**, a non-linear compound.

The configurations of glucopyranose moieties were determined to be β not only by the J value of the anomeric proton signals but also by comparison of the ^{13}C-NMR data with those of the corresponding methyl α-D- and β-D-glucosides (Yoshimoto *et al.*, 1980). The glycosidic linkage site of β-D-glucopyranose was determined to be β1→6 and C-10 based on the long-range C-H coupling between the H-1' of β-D-glucopyranosyl part and the C-10 of naphthopyrone moiety, and between the H-1" of β-D-glucopyranosyl part and the C-6' of β-D-glucopyranosyl part in an HMBC experiment. The hydroxyl group was found to be attached to C-5 according to long-range C-H coupling between hydroxy proton (δ 12.92) and C-5 in an HMBC experiment. Thus, the chemical structure of **1** was determined to be 10-[(β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl)oxy]-5-hydroxy-8-methoxy-2-methyl-4H-naphtho[1,2-*b*]pyran-4-one and was given a trivial name, isorubrofusarin gentiobioside.

We believe this to be the first report of a non-linear naphthopyrone D-gentiobioside in spite of the frequent occurrence of non-linear naphthopyrones in nature (Gorst-Allman *et al.*, 1980).

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