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MK7924, a Novel Metabolite with Nematocidal Activity from *Coronophora gregaria*

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In the course of our screening program for novel anthelmintics, a new metabolite, MK7924 (**1**), with nematocidal activity was isolated from the culture broth of *Coronophora gregaria* L2495. MK7924 (**1**) has a highly methylated polyketide skeleton with two mannoses. The structure of **1** was related to those of TMC-151s[1,2], TMC-154s[3], TMC-171s[3] and roselipins[4~6]. In this paper, we report the isolation and structure elucidation of **1**.

The producing fungus, strain L2495, was isolated from a dead twig collected in Thailand and identified as *Coronophora gregaria*[7]. *Coronophora gregaria* L2495 was inoculated onto moist rice (400 g) sterilized in five 500 ml Erlenmyer flasks. Cultivation was done under static conditions, and the resultant solid was extracted with 700 ml of 50% acetone. The extract was concentrated, then column chromatography was performed on Diaion HP-20 (Mitsubishi Chemical, Co., Japan) with methanol to give a crude oil. The crude oil was subjected to a silica gel column and eluted with a chloroform - methanol gradient. The fraction eluted with 7 : 3 (v/v) chloroform - methanol was concentrated to give 500 mg of crude residue. The crude residue was extracted with 40 ml of ethyl acetate and further purified by preparative HPLC using an ODS column (MCI-GEL ODS 1HU, 10 i.d.×300 mm, Mitsubishi Chemicals, Co., Japan; eluent, acetonitrile - water 45 : 55; flow rate, 3 ml/minute; detection, UV 215 nm) to give **1** (150 mg, Rt 16 minutes).

The physico-chemical properties of **1** are summarized in Table 1. The IR spectrum showed the presence of hydroxyl groups (3430 cm^{-1}), alkyl groups (2950 cm^{-1}) and an α,β-

Table 1. Physico-chemical properties of MK7924.

Appearance	Colorless powder
Molecular formula	$C_{34}H_{58}O_{13}$
FAB-MS (*m/z*)	
Positive:	697 $(M+Na)^+$, 713 $(M+K)^+$
Negative:	673 $(M-H)^-$
HRFAB-MS (*m/z*)	
Calcd:	697.3775
Found:	697.3755 $(M+Na)^+$
$[\alpha]_D^{25}$ (*c* 2.2, MeOH)	–9.26°
MP	101~105°C (dec)
UV λ_{max}^{MeOH} nm (ε)	233 (12,800)
IR ν_{max}^{KBr} cm^{-1}	3430, 2950, 1680, 1630, 1460, 1380,1180,1070,1020
Solubility	Soluble in methanol and chloroform; Insoluble in acetone, hexane and water

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unsaturated ketone (1630 and 1680 cm^{-1}). The molecular formula of **1** was determined to be $C_{34}H_{58}O_{13}$ by HRFAB-MS (obsd *m/z* 697.3755 calcd 697.3775 $(M+Na)^+$).

The structure of **1** was mainly elucidated by 2D NMR measurements. The ^{13}C NMR spectrum contained 34 carbon signals composed of CH_3– ×8, $-CH_2-$ ×2, $-CH_2-O-$ ×2, >CH– ×3, >CH–O– ×12, anomeric CH ×2, –CH= ×3, >C ×3 and carbonyl C ×1. To identify the carbons attached to the hydroxyl groups, chemical shifts of ^{13}C NMR spectrum in CD_3OD were compared with those in CD_3OH[8]. In the ^{13}C NMR spectrum in CD_3OH, downfield shifts of 8 carbon signals were observed, indicating that these carbons were attached to hydroxyl groups.

Analysis of the DQF-COSY spectrum revealed 4 partial structures, CH_3–CH_2–, CH_3–CH_2–CH(CH_3)–CH=, and two =CH–CH(CH_3)–CH–O– (Fig. 2). The ^{13}C NMR signals at δ 70~90 and their corresponding signals at δ 3~4 in the ^1H NMR spectrum suggested the presence of two polyalcohol moieties. It was difficult to elucidate the ^1H-^1H correlations of polyalcohol moieties by the DQF-COSY spectrum because there were many overlapping peaks. However, two sets of sequential proton networks from H-1′ (δ 4.58) and H-1″ (δ 4.34) were clarified in the HOHAHA spectrum.

The connectivities of the six partial structures, including the two polyalcohol moieties revealed by DQF-COSY and HOHAHA spectra, were determined from the ^1H-^{13}C correlations by an HMBC experiment. In the HMBC spectrum, ^1H-^{13}C long-range couplings from H_2-2 (δ 2.75 and 2.85) and H-5 (δ 6.82) to C-3 (δ 205.74) and C-4 (δ 137.06); H-7 (δ 4.14) and H-9 (δ 5.50) to C-8 (δ 132.29); H-11 (δ 4.02) and H-13 (δ 5.25) to C-12 (δ 131.60) were observed. Furthermore, as shown in Fig. 2, ^1H-^{13}C long-range couplings from anomeric protons H-1′ and H-1″ to oxygenated methane carbons C-7 and C-11, respectively, were observed. Thus, the planar structure of **1** was established.

Two polyalcohol moieties were elucidated by the coupling constants and NOESY spectrum. The large vicinal coupling constants, $^3J_{H\text{-}3',H\text{-}4'}$=9 Hz and $^3J_{H\text{-}4',H\text{-}5'}$=8 Hz, and the NOE between H-3′ and H-5′ indicated that H-3′, H-4′ and H-5′ protons were located at an axial position. Furthermore, NOE cross peaks from H-1′ to H-3′ and H-5′ were observed in the NOESY spectrum, indicating H-1′ to be an axial position. In a similar manner, H-1″, H-3″, H-4″ and H-5″ were also revealed to be axial positions. From these data, the polyalcohol moieties in **1** were deduced to be two β-mannopyranosides. β configurations of the mannopyranosides were also supported by the small coupling constants (<1 Hz) of $^3J_{H\text{-}1',H\text{-}2'}$ and $^3J_{H\text{-}1'',H\text{-}2''}$.

Table 2. NMR data of MK7924 in CD_3OD.[a]

	^{13}C	^1H
1	9.31 q	1.68 (3H, dd, *J*=9, 9 Hz)
2	31.32 t	2.75, 2.85 (2H, m)
3	205.74 s	–
4	137.06 s	–
5	149.10 d	6.82 (1H, d, *J*=11 Hz)
6	37.03 d	2.85 (1H, m)
7	88.10 d	4.14 (1H, d, *J*=12 Hz)
8	132.29 s	–
9	138.87 d	5.50 (1H, d, *J*=11 Hz)
10	35.44 d	2.75 (1H, m)
11	88.36 d	4.02 (1H, d, *J*=12 Hz)
12	131.60 s	–
13	141.00 d	5.25 (1H, d, *J*=11 Hz)
14	35.37 d	2.40 (1H, m)
15	31.30 t	1.25, 1.40 (2H, m)
16	12.59 q	0.87 (3H, m)
17	11.74 q	1.80 (3H, s)
18	16.67 q	0.90 (3H, m)
19	11.02 q	1.59 (3H, s)
20	17.89 q	0.85 (3H, m)
21	11.01 q	1.65 (3H, s)
22	21.25 q	1.00 (3H, d, *J*=8 Hz)
1'	97.21 d	4.58 (1H, s)
2'	73.04 d	3.72 (1H, m)
3'	75.82 d	3.34 (1H, m)
4'	69.15 d	3.48 (1H, ddd, *J*=9, 8, 3 Hz)
5'	78.75 d	3.05 (1H, dd, *J*=8, 5 Hz)
6'	63.25 t	3.72 (2H, m)
1"	97.36 d	4.34 (1H, m)
2"	72.84 d	3.75 (1H, m)
3"	74.88 d	3.75 (1H, m)
4"	69.34 d	3.43 (1H, ddd, *J*=9, 7, 3 Hz)
5"	77.54 d	3.42 (1H, dd, *J*=7, 5 Hz)
6"	63.15 t	3.90 (2H, m)

[a] ^1H NMR was measured at 500 MHz and ^{13}C NMR at 125 MHz.

To confirm the presence of the mannose moieties, **1** was hydrolyzed with 2 M hydrogen chloride, and the compound isolated from the hydrolysate was analyzed by HPLC using TSK gel Amide-80 column (4.6 i.d.×250 mm, Tosoh, Japan) and found to be mannopyranoside. The configuration of mannopyranoside was determined to be D form by comparing the optical rotational value with that of the authentic sample ($[\alpha]_D$+20°, authentic sample: $[\alpha]_D$+25° (*c* 0.1, H_2O)).

From the above results, the structure of MK7924 (**1**) was determined to be the novel polyketide with two β-D-mannoses as shown in Fig. 1. The structure was related to those of TMC-151s[1,2], TMC-154s[3], TMC-171s[3] and roselipins[4~6]. All of these compounds including MK7924 have a common structure of a highly methylated polyketide skeleton modified with sugar.

Fig. 1. Structure of MK7924 (**1**).

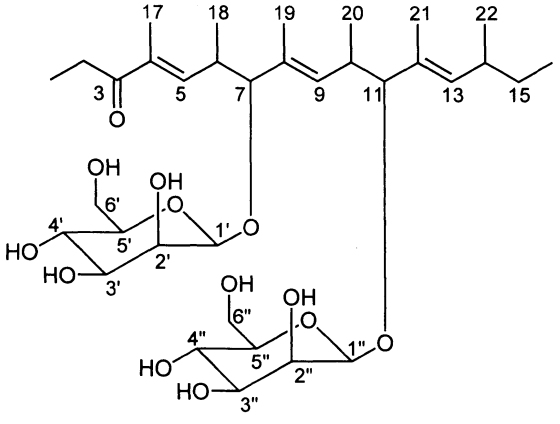
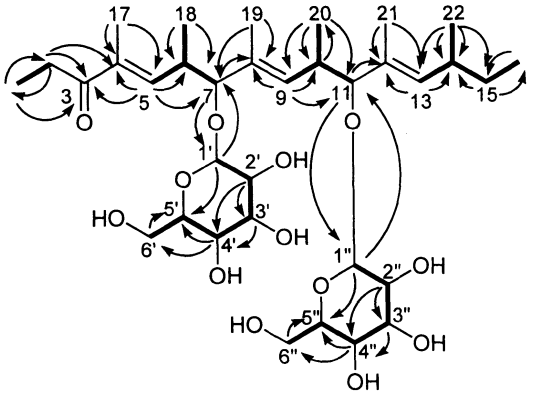


Fig. 2. DQF-COSY, HOHAHA and HMBC experiments of MK7924.



DQF-COSY, HOHAHA: —, ^{1}H-^{13}C correlations: H→C.

TMC-154s and TMC-171s are reported to have activities against several tumor cell lines[3], roselipins to be an inibitor of diacylglycerol acyltransferase (DGAT) and have antimicrobial activity[4~6]. MK7924 did not show antibacterial activity at the concentration of 100 μg/ml against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. But it had antifungal activity against *Aspergillus nigar* at the concentration of 63 μg/ml by the agar dilution method[9]. MK7924 was worthy of its marked nematocidal activity against *Caenorhabditis elegans* at 100 μg/ml by the general growth assay[7]. The effective dose against nematoda was equivalent to that of hygromycin B, but because of the structural difference between MK7924 and the known anthelmintic agents, MK7924 may be promising as a new type of anthelmintic.

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