NOTES

New Albrassitriols from *Aspergillus* sp. (FH-A 6357)†

SUSANNE GRABLEY, RALF THIERICKE* and M. ZERLIN

Hans-Knöll-Institut für Naturstoff-Forschung e.V., Beutenbergstraße 11, D-07745 Jena, Germany

AXEL GÖHRT, SIEGRID PHILIPPS and AXEL ZEECK*

Institut für Organische Chemie, Universität Göttingen, Tammannstraße 2, D-37077 Göttingen, Germany

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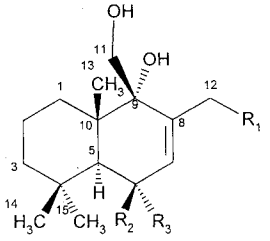
A chemical screening[2~4] guided us to a number of new secondary microbial metabolites isolated after detection on TLC with defined staining reagents. In contrast to target-oriented screening attempts our strategy is to evaluate the biological potential of isolated secondary metabolites in a subsequent step with the advantage of testing pure compounds. This supplement to biological screening strategies was sucessfully applied to various *Streptomyces* and Fungi imperfecti strains, *e.g. Aspergillus* sp. (FH-A 6357), which caused striking violet spots on TLC after staining with anisaldehyde-H_2SO_4 and brown spots with Orcinol reagent (silica gel, Rf 0.36 and 0.17, $CHCl_3$-MeOH, 9:1). In this paper we present the isolation, physico-chemical properties, structural elucidation as well as the biological activities of the detected new secondary metabolites 6-*epi*-albrassitriol (**1**) and 12-hydroxy-6-*epi*-albrassitriol (**3**).

The producing organism FH-A 6357 (deposited with the accession number DSM-7426 in the Deutsche Sammlung von Mikroorganismen (DSM), Braunschweig, Germany) has been isolated from a soil sample collected in Portugal according to common isolation procedures and was classified by means of morphological based taxonomical methods as an isolate of *Aspergillus*. On medium A (malt extract 2%, yeast extract 0.2%, glucose 1%, $(NH_4)_2HPO_4$ 0.05%, agar 2%, pH 6.0 prior to sterilization) the colonies are grey to green, while the conidia appeared to be dark brown to grey with a rough surface. Typically, the conidiophores showed phialides and metulae.

In order to examine the secondary metabolite pattern, the strain FH-A 6357 was cultivated on a rotary shaker in 300-ml Erlenmeyer flasks containing 100 ml of medium A omitting agar (medium B) at 25°C for 5 and 7 days. After filtration and adsorption of the organic compounds present in the culture medium on Amberlite XAD-16, elution with MeOH-H_2O (4:1), and a 1 to 50 concentration step, the eluates were chromatographed on TLC silica gel plates by using several solvent systems. The metabolite pattern produced was analyzed by means



		R_1	R_2	R_3
6-*epi*-Albrassitriol	(**1**)	H	OH	H
Albrassitriol	(**2**)	H	H	OH
12-Hydroxy-6-*epi*-albrassitriol	(**3**)	OH	OH	H

Table 1. Rf values, color reactions, and characterization of 6-*epi*-albrassitriol (**1**) and 12-hydroxy-6-*epi*-albrassitriol (**3**).

	6-*epi*-Albrassitriol (**1**)	12-Hydroxy-6-*epi*-albrassitriol (**3**)
Solvent systems and staining reagents:		
1-BuOH-acetic acid-H_2O (4:1:5, upper phase)	1.00	0.85
$CHCl_3$-MeOH (9:1)	0.36	0.17
Anisaldehyde-H_2SO_4	Violet	Violet
EHRLICH's reagent	Grey	Blue
Orcinol reagent	Brown	Brown
Blue tetrazolium reagent	No colorization	No colorization
Physico-chemical properties:		
Molecular formula	$C_{15}H_{26}O_3$	$C_{15}H_{26}O_4$
MW	254	270
EI-MS (70 eV; *m/z*, abundance %)	254 (8, M^+), 236 (16, M^+-H_2O), 223 (64), 205 (18), 130 (100)	270 (1, M^+), 252 (10, M^+-H_2O), 239 (54), 221 (30), 203 (30), 146 (76), 97 (100)

† Art. No. 33 on secondary metabolites by chemical screening. Art. No. 32: See ref. 1.

of color reactions carried out directly on the TLC plates by staining with different reagents (Table 1).

In 10-liter fermentors the strain *Aspergillus* sp. (FH-A 6357) was cultivated with medium B at 25°C for 5 days. Production of the new albrassitriols started about 2 to 3 days after inoculation and reached its maximum after 4~5 days. In order to purify the detected compounds from the extracellular medium the fermentation broth was separated from the mycelium by filtration. In analogy to the primary screening the organic compounds of the culture filtrate were adsorbed on Amberlite XAD-16, eluted with a 4:1 mixture of MeOH-H_2O and concentrated to dryness (yield 190 mg/liter culture broth). This dark brown oily crude product was chromatographed on a silica gel column with $CHCl_3$-MeOH (30:1) as eluant yielding two main fractions containing the desired compounds. Fraction I was further purified by gel permeation chromatography on Sephadex LH-20 (MeOH) and column chromatography on silica gel (acetone-*n*-hexane, 4:1) to yield 17.1 mg/liter pure 6-*epi*-albrassitriol. Fraction II exhibited nearly pure 12-hydroxy-6-*epi*-albrassitriol, which was rechromatographed on Sephadex LH-20 (MeOH) to yield 6 mg/liter of pure material.

The new albrassitriol derivatives are readily soluble in methanol, ethanol, acetone, and chloroform, but insoluble in *n*-hexane. Both metabolites appeared to be as colorless crystalline powders. The metabolites were characterized spectroscopically, their molecular formulae were determined by high resolution mass spectroscopy and their structures were elucidated by an analysis of the ^1H, and ^{13}C NMR spectra as well as ^1H-^1H- and ^1H-^{13}C correlation data. An independent proof of the constitution as well as additional stereochemical information resulted from X-ray analysis of 6-*epi*-albrassitriol.

The molecular formula $C_{15}H_{26}O_3$ (M^+, *m/z* 254.1882) of 6-*epi*-albrassitriol (**1**) results from a HREI-mass spectrum, which additionally show characteristic fragmentation peaks at *m/z* 236 (M^+-H_2O), 223 (M^+-CH_3O), and 130 ($C_6H_{10}O_3$). An IR spectrum showed the presence of OH-groups (3520, 3440, 3320 cm^{-1}), while absorption bands in the C=O region are missing. The optical rotation value was determined to be $[\alpha]_D^{20}$ −174.2 (*c* 0.7 in MeOH). In combination with ^1H NMR (three methyl singlets at δ 1.03, 1.11, and 1.35; a vinyl methyl (δ 1.83) coupled to a vinyl hydrogen (δ 5.52), three low field protons, seven aliphatic protons, and three OH-groups) and ^{13}C NMR data (Table 2) the constitution of the isolated compound was shown to be nearly identical with those obtained for the drimane-type sesquiterpenoid albrassitriol (**2**)[5], which has already been described as a metabolite of *Alternaria brassicae*. The main difference can be seen in the coupling constant $J_{5,6}=4.5$ Hz of **1**, which proves the *cis*-orientation of these hydrogen atoms (**2**: $J_{5,6}=10$ Hz).

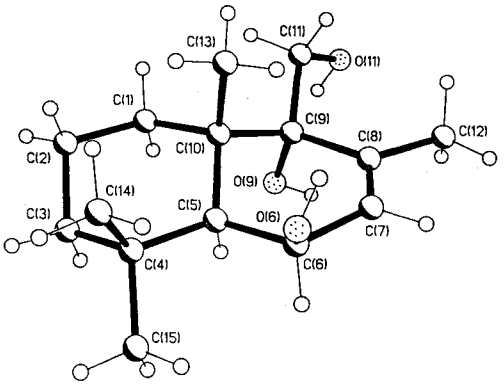
6-*epi*-Albrassitriol (**1**) can easily be crystallized by liquid-liquid diffusion of 2-propanol into a saturated chloroform solution at 8°C (mp 164°C). The structure including the relative configuration of **1** was determined on a $0.7\times0.4\times0.8$ mm^3 crystal at 20°C. As depicted in Fig. 1 the relative configuration apeared to be identical to albrassitriol (**2**) with the exception of the center of chirality at C-6. Further details of the crystal structure investigations are available on request from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen (Germany), on quoting the depository number CSD-404910, the names of the authors and the journal citation. Thus, all data are in accordance with

Table 2. ^{13}C NMR data (δ in ppm, TMS as internal standard), and ^1H NMR data of 6-*epi*-albrassitriol (**1**) and 12-hydroxy-6-*epi*-albrassitriol (**3**).

	6-*epi*-Albrassitriol (**1**)		12-Hydroxy-6-*epi*-albrassitriol (**3**)	
	^{13}C NMR (acetone-d_6, 50 MHz)	^1H NMR (acetone-d_6, 500 MHz)	^{13}C NMR (CD_3OD, 50 MHz)	^1H NMR (CD_3OD, 200 MHz)
C-1	33.2 t	1.39/1.99 (m, 1-H_2)	33.6 t	1.41/1.92 (m, 1-H_2)
C-2	19.5 t	1.45/1.67 (m, 2-H_2)	19.8 t	1.52/1.68 (m, 2-H_2)
C-3	45.2 t	1.20/ 1.28(m, 3-H_2)	45.6 t	1.22/1.30 (m, 3-H_2)
C-4	34.8 s	—	35.1 s	—
C-5	47.2 d	1.74 (d, J=4.5 Hz, 5-H)	47.5 d	1.73 (d, J=5.0 Hz, 5-H)
C-6	65.4 d	4.31 (ddd, J=5.0, 4.5, and 1.2 Hz, 6-H)	65.5 d	4.40 (dd, J=5.0 and 5.0 Hz, 6-H)
C-7	129.4 d	5.52 (dq, J=5.0 and 1.2 Hz, 7-H)	131.0 d	5.91 (dt, J=5.0 and 1.0 Hz, 7-H)
C-8	137.7 s	—	140.8 s	—
C-9	75.4 s	—	76.6 s	—
C-10	41.2 s	—	41.6 s	—
C-11	62.8 t	3.59/3.65 (J_{AB}=11.0, J_{AX}=3.5, J_{BX}=6.0 Hz, 11-H_2)	63.4 t	3.67/3.71 (J_{AB}=11.5 Hz, 11-H_2)
C-12	20.5 q	1.83 (dd, J=1.2 and 1.2 Hz, 12-H_3)	64.7 t	4.24 (s, 12-H_2)
C-13	18.9 q	1.11 (s, 13-H_3)	19.4 q	1.14 (s, 13-H_3)
C-14	25.3 q	1.35 (s, 14-H_3)	25.3 q	1.33 (s, 14-H_3)
C-15	33.4 q	1.03 (s, 15-H_3)	33.5 q	1.06 (s, 15-H_3)

Additional signals of **1**: δ_H=3.01 (d, J=5.5 Hz, 6-OH), 3.70 (s, 9-OH), 3.71 (dd, J=6.0 and 3.5 Hz, 11-OH).

Fig. 1. Perspective view of 6-*epi*-albrassitriol (**1**) with atom-numbering. The absolute configuration has not been proved.



the structure 6-*epi*-albrassitriol (**1**).

The physico-chemical data of the second metabolite showed close structural similarities with 6-*epi*-albrassitriol (**1**). In comparison, the NMR data presented the lack of both, the signal at δ_C 20.5 (C-12 in **1**) and δ_H 1.83 (12-H_3) as well as additional signals for a CH_2OH group (**3**: δ_C 64.7, C-12; δ_H 4.24, 12-H_2). In accordance to the obtained molecular formula $C_{15}H_{26}O_4$ (M^+: *m/z* 270) the isolated metabolite is 12-hydroxy-6-*epi*-albrassitriol (**3**).

The new albrassitriols have been tested in a number of different biological test systems. In the fundamental antibacterial, antifungal, herbicidal and insecticidal assays, each performed with a number of different test organisms, the new albrassitriols exhibit no significant activity. However, effects on the *de novo* formation of cholesterol in a HEP G2 cell assay[6] (40% (**1**), and 33% (**3**) inhibition at a concentration of $1.0 \cdot 10^{-8}$ mol/liter) were observed. Furthermore, 6-*epi*-albrassitriol (**1**) exhibits weak antiviral activity in *in vitro* tests against influenza A- and myxovirus (MIC 44.4 μg/ml; dosis tolerata maxima 133.3 μg/ml), while **3** was found to be inactive.

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