Four Additional Meroterpenes Produced by *Penicillium* sp Found in Association with *Melia azedarach*. Possible Biosynthetic Intermediates to Austin

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Four additional meroterpenes were isolated and identified from rice cultures of *Penicillium* sp, a fungus obtained from the root bark of *Melia azedarach*. These new compounds were named preaustinoid B2 (**1**), preaustinoid A3 (**2**), austinolide (**3**), and isoaustinone (**4**) in analogy with the formerly described compounds. The structures were identified by extensive spectroscopic studies, including 1 D and 2 D NMR spectroscopy and HRMS. Compounds **1** – **4** are probably biosynthetic intermediates to Austin.

Key words: Melia azedarach, *Penicillium*, Endophytic Fungi, Meroterpenes, Meroterpenoids

Introduction

Among the fungi isolated as endophytes from apparent health tissue of *Melia azedarach* (Meliaceae) [1], only the fungus *Penicillium* sp was found to be a producer of a series of interesting meroterpenoid compounds (compounds of a mixed terpenoid and polyketide biosynthetic origin) [2 – 4]. An analytical procedure based mainly on liquid chromatography coupled with mass spectrometry (LC/MS) was developed [5] and was shown to be efficient for the detection and identification of the known meroterpenes in fungi extract. Meanwhile, in addition to the known meroterpenes, new compounds showing similar mass spectra were detected using this approach. These compounds were isolated from the methanol extract of this microorganism and identified by HRMS and NMR spectroscopy as four novel meroterpenoids, **1**, **2**, **3** and **4** (Fig. 1). In this paper, we report on the isolation and structure elucidation of these meroterpenes. Compounds **1** – **4** are probably precursors of one of the formerly described meroterpenoids, namely austin (**5**) (Fig. 1) [6]. Biosynthetic relationships are discussed for the thirteen new meroterpenes hitherto isolated from this *Penicillium*.

Results and Discussion

Structure determination

Substances **1**, **2**, **3** and **4** were produced during cultivation of *Penicillium* sp over sterilized rice under the same conditions as those used for the production of the other meroterpenes, and isolated as amorphous solids by silica gel column and preparative thin layer chromatographic separations after their precipitation from a methanol solution of the extract from the culture.

The molecular formula $C_{24}H_{34}O_5$ for compound **1** was deduced on the basis of the ESI-MS data which detected $[M+Na]^+$ at $m/z = 425$ and $[M–H]^-$ at $m/z = 401$. The HRMS [(+)-ESI] spectrum gave $m/z =$ 425.2299 (calcd. 425.2304 for $[M+Na]^+$) confirming this molecular formula. The IR spectrum showed absorptions at 3418 cm^{-1} (hydroxyl group), 1733 and 1708 cm^{-1} (broad) (carbonyl groups). An analysis of the NMR data (Table 1) and their comparison with the formerly described compounds indicated the presence of a partial 4,4,8,10-tetramethyldecalin structure with a keto group at carbon C-3, similar to that observed in the meroterpenoids preaustinoid A (**6**) and preaustinoid B (**7**) (Fig. 1) [3]. The position of a carbonyl group at carbon C-3 was established by ^1H–^{13}C long-range correlations between two methyl groups [δ =

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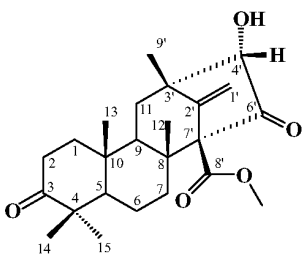
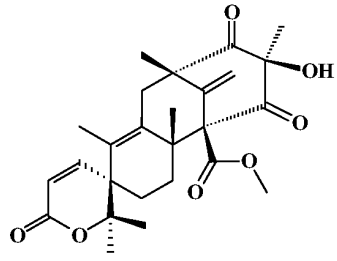
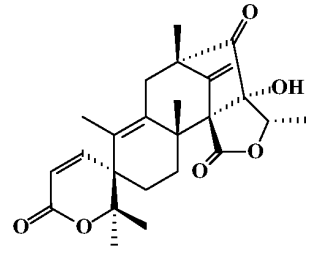


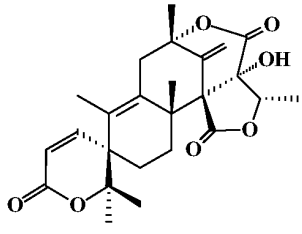
Preaustinoid B2 (**1**)



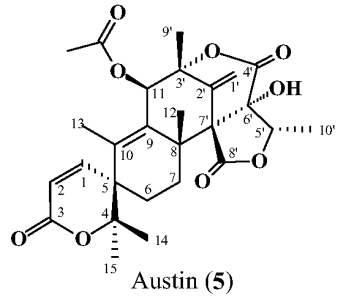
Preaustinoid A3 (**2**)



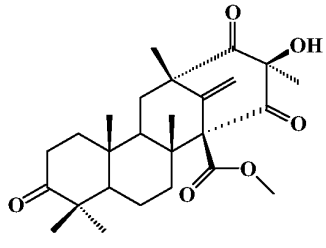
Isoaustinone (**3**)



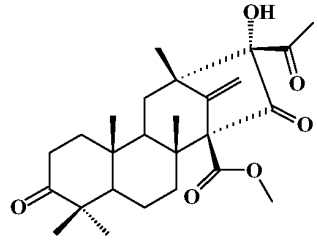
Austinolide (**4**)



Austin (**5**)



Preaustinoid A (**6**)



Preaustinoid B (**7**)

Fig. 1. Structures of compounds **1**, **2**, **3**, and **4** and other meroterpenoids reported in the literature.

1.01 (Me-14), 1.05 (Me-15)] with a carbon at $\delta = 217.2$ as shown in Fig. 2. Moreover, 2 D NMR data studies suggested a [3 : 2 : 1]-bicyclo system as partial structure for **1** like that observed in **7**. However, the methyl ketone at δ = 2.24 (Me-10′) detected for compound **7** was not observed in the ^{1}H NMR spectrum of **1**. According to HSQC data, a carbon atom at δ = 82.1 (C-4′) is correlated with the deshielded hydrogen atom at δ = 3.69, suggesting that this carbon is a tertiary carbon bearing an oxygen atom and, therefore, a hydroxyl group should be located in C-4′ position. In addition, the HMBC spectrum (Fig. 2) displayed a correlation with a methyl group at δ = 1.31 (Me-9′) with this suggesting the presence of a 2-hydroxycyclopentanone.

The stereochemistry at C-4′ was established based on the NOE observed between the methyl hydrogens at δ = 1.31 (Me-9′) with the vinyl hydrogen in the exocyclic methylene (δ = 5.11, H-1′b), and the carbinolic hydrogen H-4′ at δ = 3.69 (Fig. 3). Inversely, irradiation of the methyl hydrogens at δ = 1.20 (Me-12) caused an enhancement of the vinylic hydrogen H-1′a at $\delta = 4.86$ establishing that compound **1** has an endo-hydroxyl group in a [3 : 2 : 1]-bicyclic system forming a meroterpenoid, named preaustinoid B2, which is a new natural product.

The mass spectra obtained by both positive and negative ESI-MS for compound **2** showed peaks at *m/z* = 479 ([M+Na]$^+$) and 455 ([M–H]$^-$), and were thus consistent with the proposed molecular formula $C_{26}H_{32}O_7$

Table 1. ^{1}H NMR data (400 MHz, δ in ppm, J in Hz) for compounds **1**, **2**, **3**, and **4** in [D]chloroform (n. d. = not detected).

Pos.	**1**		**2**		**3**		**4**	
	C	H	C	H	C	H	C	H
1	38.5 t	α: 1.79 td (13.0; 13.0; 5.0) β: 1.47 dt (13.0; 5.0; 5.0)	145.8 d	6.36 d (9.8)	147.0 d	6.58 d (9.8)	146.9 d	6.63 d (10.0)
2	33.6 t	2.42 m	119.6 d	6.03 d (9.8)	119.2 d	6.02 d (9.8)	119.6 d	6.00 d (10.0)
3	217.2 s	–	164.0 s	–	n. d.	–	n. d.	–
4	48.0 s	–	85.0 s	–	85.9 s	–	85.9 s	–
5	54.2 d	α: 1.34 dd (7.0; 3.0)	45.2 s	–	45.6 s	–	46.3 s	–
6	19.8 t	α: 1.50 m	26.1 t	α: 1.57 m β: 1.59 m	26.9 t	1.62 m	28.4 t	α: 1.55 m β: 1.46 dd (14.2; 3.6)
7	31.7 t	α: 1.70 m β: 1.98 dt (13.1; 3.5; 3.5)	24.5 t	α: 2.04 dt (13.5; 3.4; 3.4) β: 2.66 td (14.7; 14.7; 3.4)	25.9 t	α: 2.57 td (13.3; 4.8) β: 1.82 dt (13.3; 4.8)	25.6 t	α: 2.95 td (13.8; 3.6) β: 1.68 dt (14.0; 3.6)
8	46.8 s	–	46.9 s	–	40.8 s	–	41.7 s	–
9	50.9 d	α: 1.19 dd (7.0; 3.2)	135.9 s	–	139.9 s	–	132.2 s	–
10	36.9 s	–	131.8 s	–	135.9 s	–	139.4 s	–
11	31.9 t	α: 1.87 dd (13.0; 3.7) β: 1.50 m	41.1 t	α: 2.22 d (14.6) β: 2.96 d (15.0)	42.4 t	α: 2.88 d (14.2) β: 2.27 dd (14.2; 1.5)	41.8 t	α: 3.15 d (16.0) β: 2.34 dd (16.0; 1.0)
12	16.5 q	1.20 s	27.0 q	1.48 s	22.2 q	1.43 s	22.7 q	1.31 s
13	16.2 q	0.88 s	15.4 q	1.49 s	15.1 q	1.56 d (1.5)	15.4 q	1.61 d (1.0)
14	20.8 q	1.01 s	22.9 q	1.22 s	23.1 q	1.24 s	22.9 q	1.18 s
15	26.7 q	1.05 s	25.9 q	1.36	25.7 q	1.37 s	25.9 q	1.32 s
1′	106.8 t	a: 4.86 s b: 5.11 s	113.9 t	a: 4.99 s b: 5.49 s	108.1 t	a: 5.25 s b: 5.23 s	115.1 t	a: 5.27 d (1.0) b: 5.55 d (1.0)
2′	149.1 s	–	143.5 s	–	146.2 s	–	133.8 s	–
3′	46.0 s	–	50.8 s	–	55.2 s	–	84.0 s	–
4′	82.1 d	3.69 s	205.9 s	–	212.5 s	–	171.1 s	–
5′	–	–	77.9 s	–	76.2 d	4.28 q (6.4)	78.9 d	4.34 q (6.0)
6′	n. d.	–	203.5 s	–	90.6 s	–	79.1 s	–
7′	71.0 s	–	71.6 s	–	66.2 s	–	63.3 s	–
8′	167.8 s	–	168.4 s	–	174.4 s	–	n. d.	–
9′	22.7 q	1.31 s	22.7 q	1.50 s	15.2 q	1.30 s	23.7 q	1.60 s
10′	–	–	16.6 q	1.19 s	12.6 q	1.28 d (6.4)	11.4 q	1.22 d (6.0)
OCH_3	52.0 q	3.66 s	52.6 q	3.76 s	–	–	–	–
OH	–	2.41 s	–	2.89 s		2.70 s		3.49 s

(HRMS ((+)-ESI): *m/z* = 479.2046; calcd. 479.2026, $[M+Na]^+$). Carbonyl groups (1732 and 1706 cm^{-1}, broad absorptions) and a hydroxyl group (3474 cm^{-1}) were evident in the IR spectrum of **2**.

The ^{1}H NMR spectrum of **2** (Table 1), analyzed with the aid of 2 D NMR data (COSY, HMBC) and by comparison with those obtained for preaustinoid A (**6**) [3] indicated a 2-hydroxy-2-methyl-1,3-dioxo partial structure (ring D). A methyl group at δ = 1.19 (Me-10′) showed ^{1}H–^{13}C long-range correlations with the carbon atoms C-5′ (δ = 77.9, ^2J), C-4′ (δ = 205.9, ^3J), and C-6′ (δ = 203.5, ^3J) in the HMBC spectrum, supporting this indication. These and other correlations can be seen in Fig. 2. The ^{1}H NMR spectrum also showed the presence of six singlets for methyl groups at δ = 1.19 (Me-10′), 1.22 (Me-14), 1.36 (Me-15), 1.48 (Me-12),

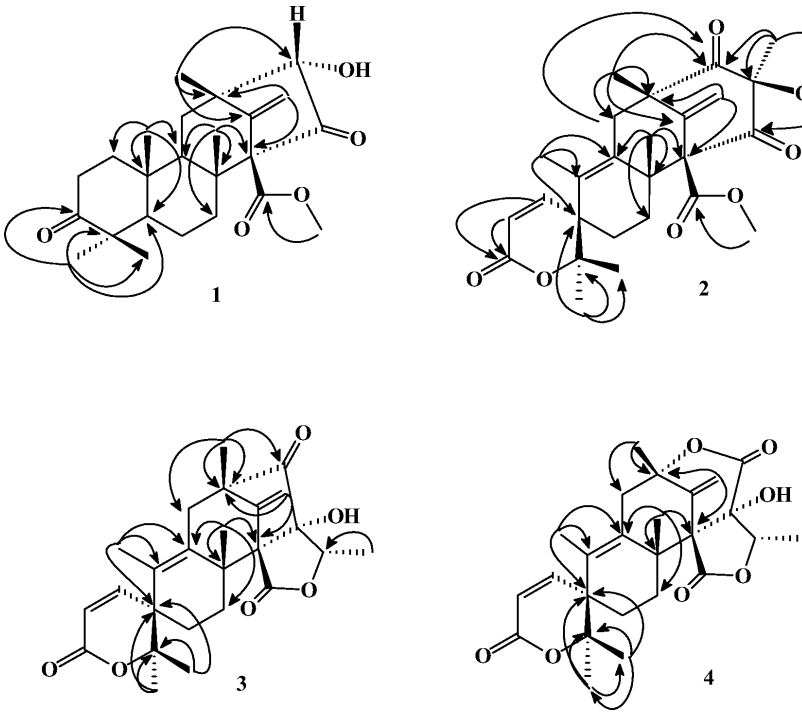


Fig. 2. HMBC correlations detected for **1**, **2**, **3**, and **4**.

1.49 (Me-13), and 1.50 (Me-9′). In addition, four signals were observed corresponding to vinylic hydrogen atoms. Two of them were attributed to the exocyclic methylene at $\delta = 4.99$ (H-1′a) and 5.49 (H-1′b). The remaining were related to a pair of deshielded hydrogen atoms at $\delta = 6.36$ (d, 9.8 Hz, H-1) and 6.03 (d, 9.8 Hz, H-2) suggesting an α,β-unsaturated δ-lactone. A correlation between these hydrogens with a carbonylic carbon atom at δ = 164.0 (C-3) in the HMBC spectrum confirmed the presence of this α,β-unsaturated δ-lactone unit. Moreover, the long-range correlations between the methyl groups at δ = 1.22 (Me-14) and 1.33 (Me-15) with the carbon atoms at δ = 85.0 (C-4, ^2J) and 45.2 (C-5, ^3J) as well as that observed between the methyl group at δ = 1.49 (Me-13) with C-5 (^3J) suggested a spiro connectivity between rings A and B (Fig. 2). The ^{13}C NMR spectrum revealed another pair of sp^2 carbon atoms at δ = 131.8 and 135.9, which were attributed to carbons C-10′ and C-9′, respectively, after observation of the correlation of the methyl groups Me-12 and Me-13 with these carbon atoms.

Final elucidation of the structure for **2** came from NOE experiments. Irradiation of H-1′a at $\delta = 4.99$ produced an enhancement of the signals for hydrogens H-1′b (δ = 5.49) and Me-12 (δ = 1.48), while the hydrogen H-1′b at δ = 5.49 caused an increase of the signals at δ = 4.99 and 1.50 (H-1′a and Me-9′, respectively). These and other correlations are shown in Fig. 3. Consequently, the meroterpenoid **2** is a new natural product and was named preaustinoid A3 out of having the same D ring observed in meroterpenoid **6**.

The analysis of the molecular formula of **3** ($C_{25}H_{30}O_6$, 426 Da), deduced from the mass spectra obtained by ESI-MS ($[M+Na]^+$ at *m/z* = 449, $[M+H]^+$ at *m/z* = 427 and $[M–H]^-$ at *m/z* = 425) and confirmed by HRMS ((+)-ESI: *m/z* = 449.1943; calcd. 449.1940 for $[M+Na]^+$) indicated that the compound contains 11 double bond equivalents (DBE), one more than calculated for compound **2**. Comparison of their NMR spectra revealed that compounds **2** and **3** are two closely related meroterpenes, with identical A, B, and C rings. The ^{1}H NMR spectrum of **3** (Table 1) contains a pair of doublets corresponding to *cis*-olefinic hydrogens at δ = 6.02 (d, J = 9.8 Hz) and 6.58 (d, J = 9.8 Hz) for H-2 and H-1, respectively, in ring A, forming an α,β-unsaturated δ-lactone. The presence of a spiro-system was confirmed by ^{1}H–^{13}C long-range correlations observed in the HMBC spectrum. The methyl groups at δ = 1.24 (Me-14), 1.37 (Me-15), and 1.56 (Me-12) showed correlation with a carbon atom at δ = 45.6 (C-5). Moreover, the pair of sp^2 carbons in ring B

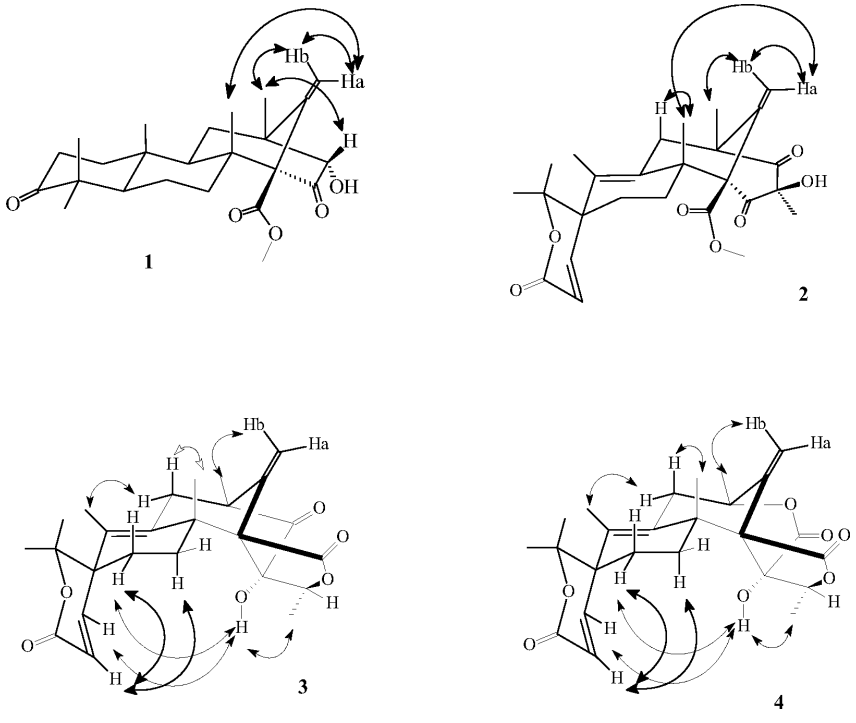
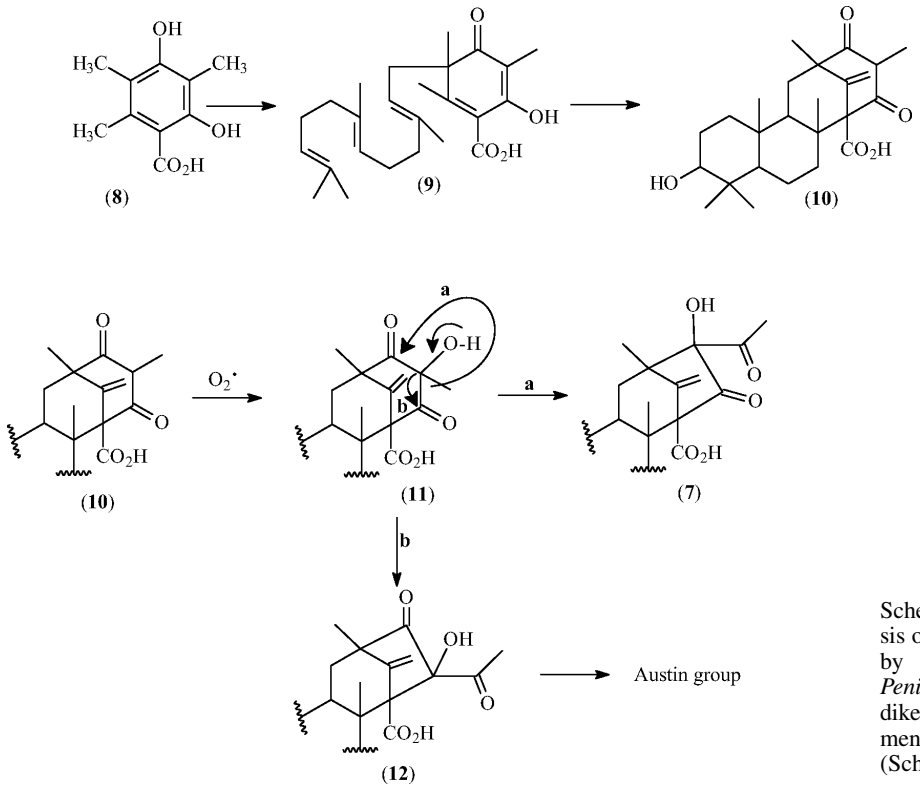


Fig. 3. Spatial correlations suggested for **1**, **2**, **3**, and **4** based on NOE difference spectra.

at δ = 135.9 (C-10) and 139.9 (C-9) was established through correlations with the methyl groups Me-13 (δ = 1.56) and Me-12 (δ = 1.43), as well as the vinylic hydrogens of the exocyclic methylene group in ring C by their correlations with the quaternary carbon atoms at δ = 55.2 (C-3′) and 66.2 (C-7′). These and other correlations are visualized in Fig. 2.

However, the expected ^{1}H (δ = 3.70 *ca.*, s) and ^{13}C (δ = 53.0 *ca.*) NMR signals that characterize the carbomethoxyl group in **1** and **2** are not present in the spectra of **3**. Furthermore, the remaining keto group detected at δ = 212.5 (C-4′) and a carbonyl group at δ = 174.4 (C-8′) suggested the presence of a γ-lactone system in meroterpene **3**. The positioning of the keto carbonyl in ring D was confirmed by the observed correlation between the methyl group at δ = 1.30 (Me-9′) with this carbon atom. The doublet at δ = 1.28 was attributed to methyl group Me-10′ (d, J = 6.4 Hz) which resulted from the coupling with a deshielded carbinolic hydrogen atom at δ = 4.28 (q, J = 6.4 Hz, H-5'). This suggested the presence of a γ-lactone forming the ring E in meroterpene **3**. Final confirmation of the structure of **3** was obtained from a NOESY spectrum with correlations as presented in Fig. 3. A correlation between the hydrogens H-1′b (δ = 5.23) and Me-9′ (δ = 1.30) indicated that these groups are on the same face of the molecule. The correlation observed between the hydroxylic hydrogen atom at δ = 2.70 and H-7α at δ = 2.57 suggested that the hydroxyl group has an α-configuration. Moreover, the configuration of the spiro carbon C-5 was established by the correlation detected for the hydrogen atoms H-1 (δ = 6.58) and H-7α (δ = 2.57). Thus the meroterpene **3** was named isoaustinone and is a new natural product.

The ESI mass spectra of **4** showed the ions $[M+H]^+$ detected at *m/z* = 443, $[M+Na]^+$ at *m/z* = 465, and $[M–H]^-$ at *m/z* = 441, from which the molecular formula $C_{25}H_{30}O_7$ (442 Da) was deduced and confirmed by HRMS ((+)-ESI: *m/z* = 523.5734; calcd. 523.5944, $[M+Na]^+$). The IR spectrum showed absorptions at 3126 (hydroxyl group), 1777, 1731, 170 (carbonyl groups), and 1650 cm^{-1} (vinyl group). Comparison of these data with those obtained for compound **3** revealed a difference of 16 a. m. u which corresponds to an additional oxygen atom in **4**. The ^{1}H, ^{13}C and 2 D NMR spectra showed that compounds **3** and **4** are very similar, except for the C-3′ and C-4′ positions. A carbonyl lactone was observed in the ^{13}C NMR spectrum (Table 1) at δ = 171.1 (C-4′) rather than the keto group detected for meroterpenoid **3** in this position. Moreover, the C-3′ chemical shift at δ = 84.0 suggested a quaternary oxygenated carbon which was confirmed



Scheme 1. Proposed biosynthesis of the meroterpenes produced by species of *Aspergillus* and *Penicillium*. Formation of the 1,3 diketone and α-ketol rearrangements follow by paths *a* and *b* (Scheme 2).

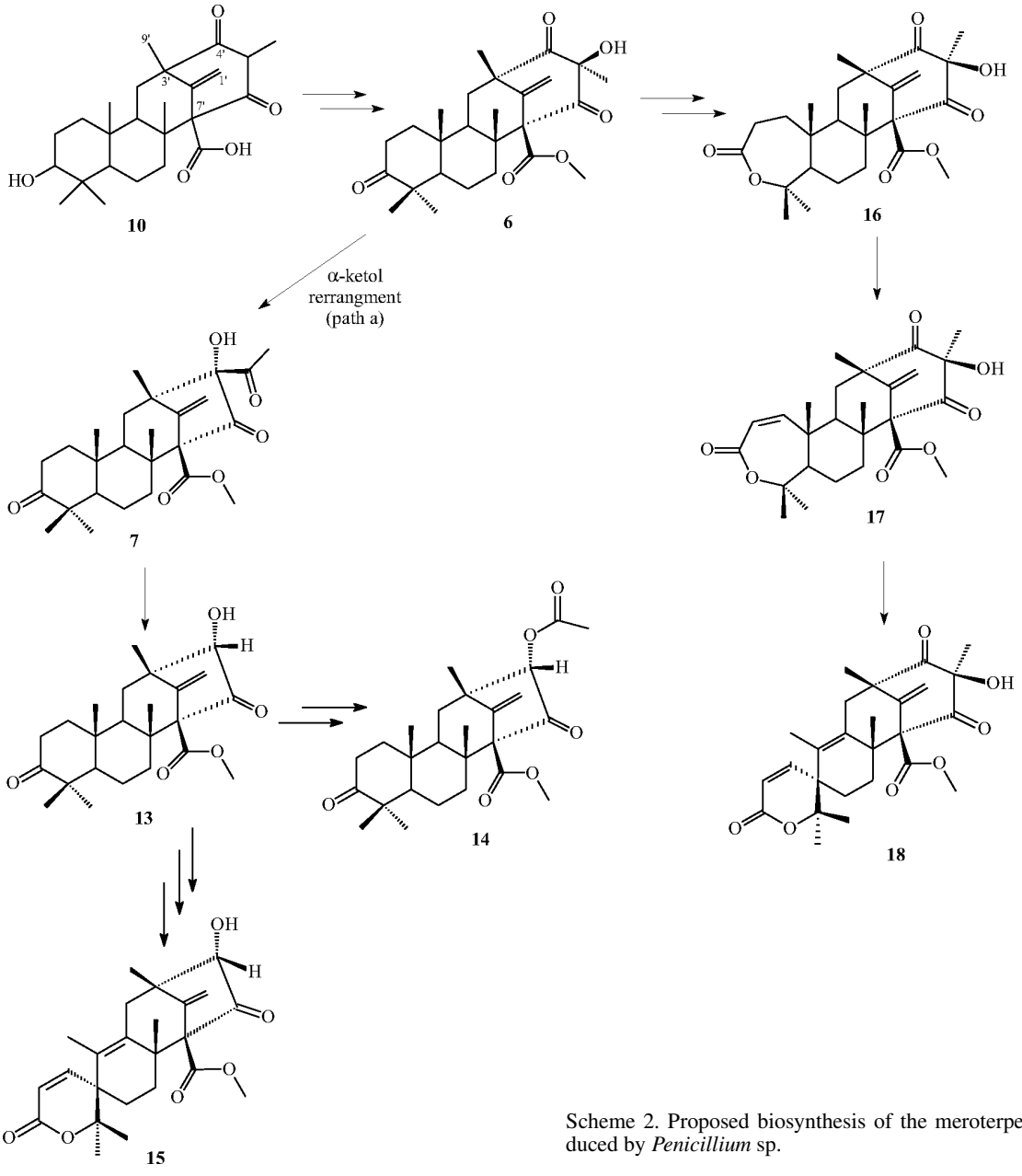
through the long-range correlations of the hydrogens H-1′a, H-1′b and Me-9′ (δ = 5.55, 5.27, and 1.60, respectively) with this carbon atom.

According to the data obtained from the NOE studies (NOESY, gNOESY), compound **4** shows the same relative stereochemistry at the stereogenic centers of its molecule. Irradiation of H-1 (δ = 6.63) produced an enhancement of the signals at δ = 6.0, 1.55, and 2.95, respectively (for H atoms H-2, H-6α, and H-7α). Irradiation of a doublet at δ = 1.61 (H-13) resulted in the enhancement of the signal for H-11 (δ = 3.15), which corroborated our attribution of the α-configuration to this hydrogen atom. Moreover, the NOE observed between H-12 (δ = 1.31) and H-11β (δ = 2.34) supported this supposition. Irradiation of H-9′ at δ = 1.60 produced an enhancement of H-1′b at δ = 5.55, while irradiation of H-12 (δ = 1.31) resulted in an enhancement of H-1′a at δ = 5.27. In addition, irradiation of the hydroxyl hydrogen atom at δ = 3.49 led to an enhancement of hydrogen atoms H-7α (δ = 1.55) and H-1 (δ = 6.63), indicating that they are on the same face of this meroterpene. These correlations are shown in Fig. 3. Therefore, the meroterpenoid **4** is a new natural product named austinolide and probably is biosynthetically derived from the meroterpenoid **3**, as shown in the following discussion.

Biosynthesis considerations

Our interest in these compounds began with the isolation of preaustinoid A (**6**) and preaustinoid B (**7**) from *Penicillium* sp [3]. Other clearly related structures of these metabolites from this fungus [4, 7] suggested that these compounds could be precursors of the meroterpenoid austin (**5**). The observation of the four compounds reported in the present paper seems to complete a list of thirteen meroterpenes produced by this *Penicillium*.

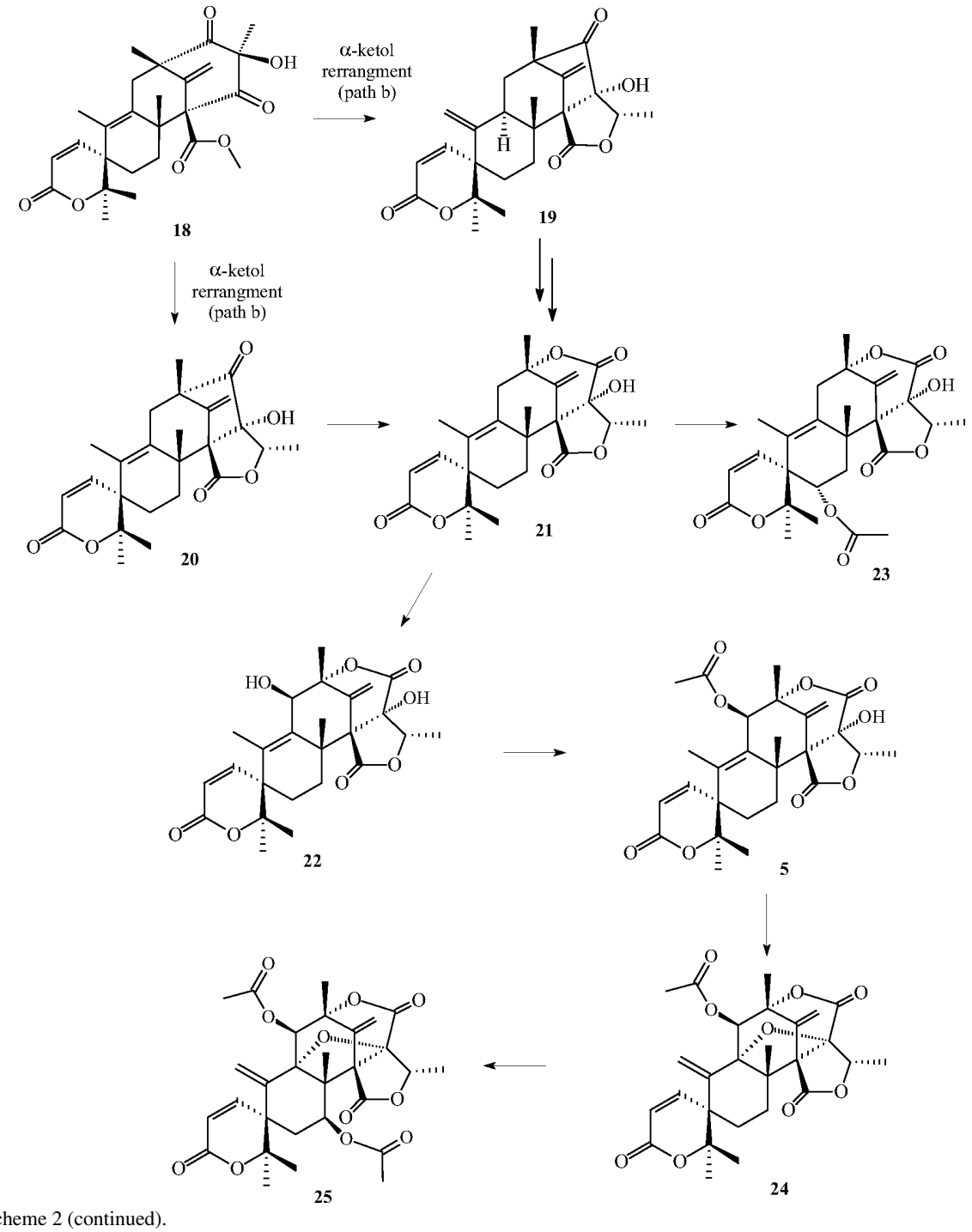
Studies on the biosynthesis of meroterpenes produced by *Aspergillus* and *Penicillium* suggested that the compounds in this group of metabolites are formed by di-C-alkylation of 3,5-dimethyl-orsellinic acid (**8**) by farnesyl pyrophosphate [2, 8, 9]. Further alkylation and cyclization would result in the formation of a 1,3-diketone (**10**), which has been postulated to be a precursor of the meroterpenes of the austin group (Scheme 1) [9]. Further information on the mechanisms involved in the drastic modifications of the tetraketide and farnesyl-derived modifications have



Scheme 2. Proposed biosynthesis of the meroterpenes produced by *Penicillium* sp.

been provided by extensive studies with ^{13}C, ^{2}H, and ^{18}O labeled precursors [8]. The results of incorporation of $^{18}O_2$ into austin indicated a modification mechanism in which the orsellinate moiety undergoes a ring contraction *via* an α-ketol rearrangement followed by Baeyer-Villiger-type oxygen insertion to form the δ-lactone. ^{18}O and ^{2}H labeling has also provided information on the mechanism of formation of the spirolactone systems observed in andibenins and austin (**5**) [2]. These results have shown that the C-3 lactone function must be formed by a biological Baeyer-Villiger-type oxidation of the corresponding 3-keto precursor.

The structures of the compounds obtained from the cultures of *Penicillium* sp provide additional support for this general route. The occurrence of these meroter-



Scheme 2 (continued).

penes corroborates the results of earlier studies of this biosynthesis and also demonstrates that the α-ketol rearrangement in diketone **10** (paths a and b, Scheme 1) is not regioselective in *Penicillium* sp. The meroterpenes preaustinoid B (**7**) is formed *via* an α-ketol rearrangement by path a (Scheme 2) and through subse-

quent modifications yields the meroterpenes preaustinoid B2 (**13**), preaustinoid B1 (**14**) and austinoneol (**15**).

On the other hand, preaustinoid A (**6**) undergoes modifications in its ring A producing the meroterpenes preaustinoid A1 (**16**), preaustinoid A2 (**17**), and preaustinoid A3 (**18**). The latter produces the meroterpenes neoaustin (**19**) and isoaustinone (**20**) by an α-ketol rearrangement in path b (Scheme 2). Subsequent oxidations in the tetraketide portion give the meroterpene austinolide (**21**).

Successive oxidation process and introduction of the acetyl group occur to give the compounds austinol (**22**), isoaustin (**23**), and austin (**5**). The occurrence of a furanone ring in the meroterpenes dehydro-austin (**24**) and acetoxy-dehydro-austin (**25**) probably could be the result of a radical mechanism.

It therefore appears that α-ketol rearrangements and Bayer-Villiger oxidations are the main enzymatic reactions in these biosynthetic processes. Surprisingly, these reactions also occur during the biosynthesis of limonoids, which are endogenous compounds produced by *Melia azedarach*, the host plant from which the meroterpene producing fungus was isolated.

Experimental Section

General experimental procedures

Optical rotations were measured on a Perkin Elmer 241 polarimeter. UV spectra were obtained in $CHCl_3$ solution on a Hewlett Packard 8452-A spectrophotometer, and IR spectra were measured with a Bomen MB-102 spectrophotometer on KBr pellets. Low-resolution ESI-MS data were acquired in positive and negative ion mode, using a MICROMASS QUATTRO-LC instrument equipped with an ESI/APCI "Z-spray" ion source. High-resolution ESI-ToF MS data were acquired using a WATERS MicrOTOF equipment. 1H and ^{13}C NMR experiments were recorded on a BRUKER DRX-400 spectrometer using [D]chloroform as solvent and TMS as the internal standard.

Plant material

Cortex roots of *Melia azedarach* were collected at campus of the Federal University of São Carlos, São Carlos, Brazil. A voucher specimen has been deposited in the Herbarium of the Department of Botany at this University.

Isolation of the microorganism

The general procedure adopted has been described previously in ref. [1]. Immediately after collection, the root bark was separated mechanically from the xylem and washed with water followed by ethanol and then sterilized with 11 % aqueous sodium hypochlorite for 1 min. The material was then deposited on a Petri dish containing PDA (potato-dextrose-agar) medium and incubated in the dark at 25 °C for one week. *Penicillium* sp was isolated by replication and grew as a bluish colored culture. The fungus was identified and deposited at the Laboratório Bioquímica Micromolecular of Microorganisms (LaBioMMi) of the Departamento de Química at Universidade Federal de São Carlos, São Carlos, Brazil (number 24).

Rice culture of Penicillium sp and isolation of the meroterpenes

Fifty Erlenmeyer flasks (500 mL) containing about 90 g of rice ("Uncle Ben's") and 75 mL of distilled water per flask were autoclaved twice at 121 °C for 40 min. A small disc of the PDA medium from the Petri dish containing mycelium of *Penicillium* sp was transferred under sterile conditions to 47 of the 50 Erlenmeyer flasks containing sterilized rice. Three flasks were kept for control purposes. After 20 days of growth, the water that had accumulated in the flasks was separated by filtration from the fungal biomass. About 150 mL of methanol was added to each flask and the mixtures were allowed to stand for 5 h, after which they were filtered by gravity. The methanol was evaporated under reduced pressure, producing a yellowish residue (6 g), which was chromatographed on silica gel CC and eluted with a hexane to methanol gradient. The medium-polarity fractions eluted with dichloromethane/1 % methanol were repeatedly chromatographed on silica gel columns and subjected to preparative TLC using hexane/dichloromethane/acetone (37 : 57 : 6) and hexane/ethyl acetate (50 : 50) to afford preaustinoid B2 (**1**), preaustinoid A3 (**2**), isoaustinone (**3**), and austinolide (**4**).

*Preaustinoid B2 (**1**):* M. p. 178 – 181 °C. – UV/vis (CH_2Cl_2): λ_{max} = 218 nm. – $[\alpha]_D^{25}$ = +90.0 (c = 1.0, $CHCl_3$). – IR (KBr): ν = 3418 (OH), 2920, 2850, 1733 (C=O), 1708 (C=O), 1458 cm^{-1}. – ^{1}H NMR (400.12 MHz, $CDCl_3$, 25 °C, TMS): see Table 1. – ^{13}C NMR data obtained by PENDANT [10], HSQC and HMBC (100.03 MHz, $CDCl_3$): see Table 1. – MS [(–)-ESI, daughter ions, 30 eV]: *m/z* (%) = 401 (100) $[M–H]^-$, 383 (18), 369 (60), 341 (21), 75 (51). – HRMS [(+)-ESI]: *m/z* = 425.2299 (calcd. 425.2304 for $C_{24}H_{34}O_5Na$, $[M+Na]^+$).

*Preaustinoid A3 (**2**):* M.p. 209 – 212 °C. – UV/vis (CH_2Cl_2): λ_{max} = 219, 261(s) nm. – $[\alpha]_D^{25}$ = +290.2 (c = 0.22, $CHCl_3$). – IR (KBr): ν = 3474 (OH), 2989, 2922, 2850, 1732 (C=O), 1706 (C=O), 1644, 1230, 1015, 923 cm^{-1}. – ^{1}H NMR (400.12 MHz, $CDCl_3$, 25 °C, TMS): see Table 1. – ^{13}C NMR data obtained by PENDANT [10], HSQC and HMBC (100.03 MHz, $CDCl_3$): see Table 1. – MS [(–)-ESI, daughter ions, 30 eV]: *m/z* (%) = 413 (100) $[M–H]^-$, 381

(10), 353 (15), 59 (10). – HRMS [(+)-ESI]: *m/z* = 479.2044 (calcd. 479.2046 for $C_{26}H_{32}O_7Na$, $[M+Na]^+$).

*Isoaustinone (**3**):* M. p. 299 – 303 °C. – UV/vis (CH_2Cl_2): λ_{max} = 218, 263 (s) nm. – $[\alpha]_D^{25}$ = +175.3 (*c* = 1.13, $CHCl_3$). – IR (KBr): ν = 3489 (OH), 2921, 2851, 1753 (C=O), 1709 (C=O), 1455, 1377 cm^{-1}. – ^1H NMR (400.12 MHz, $CDCl_3$, 25 °C, TMS): see Table 1. – ^{13}C NMR data obtained by PENDANT [10], HSQC and HMBC (100.03 MHz, $CDCl_3$): see Table 1. – MS [(–)-ESI, daughter ions, 30 eV]: *m/z* (%) = 425 (100) $[M–H]^-$, 397 (87), 381 (28), 363 (70), 353 (41), 337(15), 319 (38), 259 (57), 195 (56), 151 (29). – HRMS [(+)-ESI]: *m/z* = 449.1943 (calcd. 449.1940 for $C_{25}H_{30}O_6Na$, $[M+Na]^+$).

*Austinolide (**4**):* M. p. 277 – 281 °C. – UV/vis (CH_2Cl_2): λ_{max} = 219, 262 (s) nm. – $[\alpha]_D^{25}$ = +261.0 (*c* = 0.39, $CHCl_3$). – IR (KBr): ν = 3326 (OH), 2923, 2850, 1777 (C=O), 1731 (C=O), 1708 (C=O), 1650, 1398 cm^{-1}. – ^1H NMR (400.12 MHz, $CDCl_3$, 25 °C, TMS): see Table 1. – ^{13}C NMR data obtained by PENDANT [10], HSQC and HMBC (100.03 MHz, $CDCl_3$): see Table 1. – MS [(–)-ESI, daughter ions, 30 eV]: *m/z* (%) = 441 (100) $[M–H]^-$, 353 (52), 341 (68), 297 (97), 282 (71), 117 (63), 73 (21). – HRMS [(+)-ESI]: *m/z* = 523.5734 (calcd. 523.5904 for $C_{27}H_{32}O_9Na$, $[M+Na]^+$).

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