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Multiflorane triterpenoid esters from pumpkin. An unexpected extrafolic source of PABA ☆

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Abstract

The seeds of pumpkin and squash contain relatively large amounts of two multiflorane triterpenoids (**1a**, **2a**) esterified with PABA, a folic acid constituent found for the first time in secondary plant metabolites. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Cucurbita pepo*; *Cucurbita maxima*; Cucurbitaceae; Triterpenoids; Multifloranes; *p*-Aminobenzoates

1. Introduction

PABA (*para*-aminobenzoic acid) is a constituent of tetrahydrofolic acid, a versatile carrier of one-carbon fragments required for the synthesis of important primary metabolites like thymidylate, purines, and certain amino acids (e.g. methionine from homocysteine) (Blakley, 1969). Interference with the incorporation of PABA into dihydropteroic acid, the immediate precursor of folic acid, underlies the antimicrobial activity of sulfa drug (Woods, 1962) and there has been great interest in the chemistry and biochemistry of PABA. *Ortho*- and *para*-aminobenzoates are both derived from chorismate through the agency of related enzymes (Haslam, 1993). While *o*-aminobenzoate (=anthranilate) is firmly enshrined in the mainstream of secondary metabolism, no secondary plant product containing PABA has so far been described (Dictionary of Natural Products) and this conservative use of PABA highlights its relevance to primary metabolism. We report now that the seeds of pumpkin, a popular snack and the source of an edible and medicinal oil, contain relatively large amounts (0.02–0.08%) of two triterpenes esterified with PABA.

2. Results and discussion

Despite the dietary and medicinal interest in pumpkin seeds oil (Bombardelli, & Morazzoni, 1997), a constituent of non-proprietary drugs for the treatment of benign prostate hyperplasia (BHP) (Bombardelli, & Morazzoni, 1997), relatively little is known on its terpenoid constituents, presumably because of the difficulty to separate lipophilic products from a fatty matrix. After considerable experimentation, we found that partition of acetone extracts from the seeds of pumpkin (*Cucurbita maxima* Duch.) or zucchini (*C. pepo* L.) between hexane and acetonitrile (Berge, & Roberts, 1979) could remove most of the fats, leaving a terpenoid fraction whose major constituent was the allylic alcohol **1a**. This compound was isolated as an unstable greenish oil. Further purification by RP-column chromatography gave the dehydrated product **2a**,

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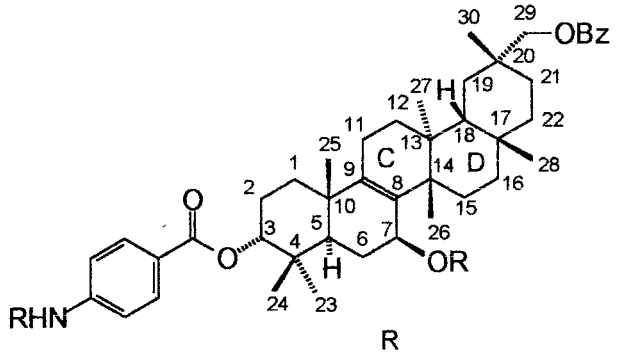
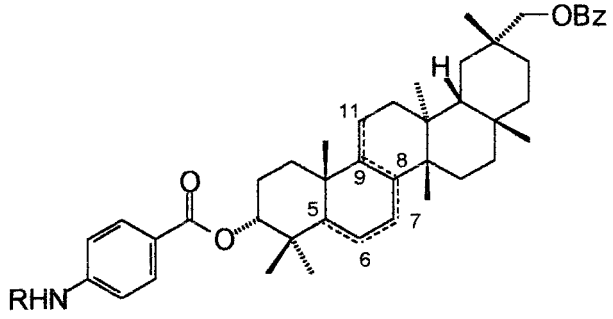


R

1a H

1b Ac



R Δ

2a H 7, 9 (11)

2b Ac 6, 8

2c Ac 7, 9 (11)

2d Ac 5, 7, 9 (11)

identical to a compound obtained from chromatographic fractions less polar than those containing **1a**. (*see infra*). HPLC on silica gel could eventually remove the coloured impurities, and afforded a crystalline material.

MS established a molecular weight of 681 for **1a**, corresponding to the molecular formula $C_{44}H_{59}NO_5$. The ^{1}H-NMR spectrum in $CDCl_3$ disclosed the presence of a trioxygenated triterpenoid core carrying one benzoyl moiety, one free hydroxyl and one *para*-substituted benzoyl ester group. The large chemical shift difference between the external lines of the AA′BB′-spin system ($\Delta\delta = 1.71$ ppm) suggested the presence of an electron-donating amino group *para* to an electron withdrawing carbonyl (Pretsch, Seibl, Simon, & Clerc, 1989). The instability of **1a** in $CDCl_3$ and its only limited stability in C_6D_6 prevented further experiments. The obtaining of derivatives more stable and amenable to a detailed spectroscopic investigation was thus pursued. Reaction with Ac_2O in pyridine gave the crystalline amide **1b** which showed an enhanced stability in C_6D_6. The conversion of the primary amino group to an amide was evidenced by the detection of one D_2O-exchangeable amide broad singlet at δ 8.19 (Table 1), showing HMBC correlations with the signal of C3/C5 of the *p*-substituted benzoyl moiety. This assignment was further confirmed by a decrease (1.31 ppm) of the $\Delta\delta$ between the external lines of the AA′BB′ system (Pretsch et al., 1989) and by diagnostic IR bands at 3374, 1690 and 1526 cm^{-1}. Compound **1b** showed well-resolved ^{1}H- and ^{13}C-NMR spectra, which could be all assigned in C_6D_6 using 2D techniques (HMQC, HMBC) and NOE-difference spectroscopy. Analysis of the scalar and dipolar H–H connectivities and the ^{1}H–^{13}C correlations established the constitution and stereochemistry of the terpenoid core (Δ^8-multiflorane (= D:C-friedo-oleanane)-3α,7β,29-triol), and located the ester groups at C-3 (*p*-aminobenzoyl), C-7 (acetyl) and C-29 (benzoyl). The configuration at C-3 was assigned on the basis of diagnostic NOE-correlations of H-3 (24-methyl) and the *ortho*-protons of the PABA moiety (23-methyl and 27-methyl), while the β-configuration of the 7-acetyl was shown by the NOE-correlations H-7/H-5, and H-7/H-27. Finally, the α-orientation of the 29-benzoyloxy group was evidenced by the NOE-correlations H-29a,b/H-27 and H-27/*ortho* benzoate protons. The natural allylic alcohol should thus have the structure represented by **1a**.

Also the diacetate **1b** was not stable in $CDCl_3$. After 24 h at room temp., **1b** was quantitatively converted to a ca. 10:1 mixture of the $\Delta^{6,8}$- and $\Delta^{7,9(11)}$-dienes **2b** and **2c**. The $\Delta^{6,8}$-diene was then slowly oxidised in the air to the $\Delta^{5,7,9(11)}$-triene **2d**. Reaction of **1a** with triethylsilyl chloride in DMF gave the $\Delta^{7,9(11)}$-diene **2a** as the only reaction product. The formation of only the $\Delta^{7,9(11)}$-isomer in the dehydration of **1a** is remarkable, and stands in sharp contrast to the results observed with its corresponding acetate, which gave mainly the isomeric $\Delta^{6,8}$-diene. The easy conversion of the allylic alcohol **1a** to the diene **2a** under RP-chromatographic conditions or upon treatment with silylating reagents makes it possible that this diene is an artefact of isolation and/or purification procedures.

Compounds **1a** and **2a** could also be isolated from the seeds of squash (*C. pepo* L.), buffalo gourd (*C. foetidissima* H.B.K.) (Thompson, 1990) and cucumber (*Cucumis sativus* L.), suggesting that these multiflorane *p*-aminobenzoates might be of widespread occurrence within pumpkins and related plants. The wide distribution of **1a** and its instability raise the possibility that isokarounidiol, the diol corresponding to **2b** and the only naturally occurring triterpene with the $\Delta^{6,8}$-diene system (Akihisa, Kokke, Kimura, & Tamura, 1993), is actually an isolation artefact. A series of multiflorane dienes and trienes obtained after saponification of seed

Table 1
^{1}H NMR data for **1a**, **1b**, **2a** (C_6D_6) and **2b**, **2d** ($CDCl_3$) (500 MHz for **1a** and **1b**; 400 MHz for **2a**, **2b** and **2d**)[a]

Proton	**1a**	**1b**[b]	**2a**	**2b**	**2d**[c]
1α	*	1.60 m	1.97 m	1.75–1.45 m	*
1β	*	1.20 m	1.43 m		*
2α	*	1.80 m	1.99 m	1.95 m	*
2β	*	1.70 m	1.84 m	1.83 m	*
3	5.13 br s	5.04 br s	5.16 br s	4.88 br dd	4.92 br d
5	1.89 dd	2.00 dd	2.08 m	2.53 br dd	–
6α	*	2.64 ddd	2.00 m	5.75 br dd	5.86 br d
6β	*	1.63 m	2.15 m	–	–
7	4.35 m	5.82 br dd	5.58 br d	6.10 br dd	5.66 br d
11α	*	2.03 m	5.27 br d	2.10 m	5.40 br ddd
11β	*	1.80 m	–	1.85 m	–
12α,β	*	1.50–1.40 m	2.11 br dd 1.78 br d	1.57 m 1.38 m	*
15α,β	*	1.70–1.40 m	1.75 m 1.45 m	1.75–1.45 m	*
16α	*	1.45 m	1.75 m	1.67 m	*
16β	*	1.65 m	1.50 m	1.48 m	*
18	*	1.65 m	1.62 br d	1.64 m	1.72 m
19α	*	1.70 m	1.82 br d	1.70 m	*
19β	*	1.40 m	1.55 dd	1.41 m	*
21α,β	*	1.55 m	1.52 m 1.42 m	1.54 m	*
22α	*	1.65 m	1.76 m	1.64 m	*
22β	*	0.84 m	0.83 br d	1.03 m	*
23	1.04 s	0.99 s	0.98 s	0.98 s	1.12 s
24	0.84 s	0.76 s	0.89 s	1.08 s	1.27 s
25	1.00 s	1.04 s	1.02 s	0.80 s	1.24 s
26	1.43 s	1.38 s	1.08 s	1.18 s	1.04 s
27	1.05 s	0.93 s	1.04 s	1.08 s	0.91 s
28	1.13 s	1.10 s	1.06 s	1.12 s	1.12 s
29a	4.41 d	4.33 d	4.31 d	4.08 s	4.31 d
29b	4.08 d	4.12 d	4.22 d		4.06 d
30	1.15 s	1.14 s	1.13 s	1.14 s	1.16 s
OBz	8.21 AA′ 7.10 BB′ 7.14 C	8.15 AA′ 7.06 BB′ 7.15 C	8.27 AA′ 7.10 BB′+C	8.04 AA′ 7.45 BB′ 7.56 C	7.97 AA′ 7.42 BB′ 7.54 C
OPABA	8.20 AA′ 6.49 BB′	8.31 AA′ 7.91 BB′	8.16 AA′ 6.79 BB′	7.97 AA′ 7.59 BB′	7.78 AA′ 7.46 BB′
NH_2 (NH)	*	7.62 br s	3.30 br s	7.69 br s	8.62 br s
N-Ac	–	1.68 s	–	2.20 s	2.20 s
OAc	–	1.82 s	–	–	–

[a] *J* values (in Hz): for **1a**: 5,6a(b)=13, 2; 29a,b=11. For **1b**: 5,6a=2; 5,6b=14; 6a,b=13; 6a,7=6b,7=8; 29a,29b=11. For **2a**: 6,7=5; 11,12a=5; 12a,b=12.5; 18,19b=8; 19a,b=15; 22a,22b=13; 29a,b=11. For **2b**: 2a,3=2b, 3=3; 5,6=5,7=3; 6,7=10. For **2d**: 2,3=3.5; 6,7=6; 11,12a,b=5.5, 2.2; 29a,29b=10.5.

[b] Selected NOEs: H-7 with H-5, H-6α and H-27; H-23 with H-5, H-6α, H-24 and AA_{PABA}'. H-24 with H-2β, H-3, H-23 and H-25. H-25 with H-1β, H-2β, H-6β, H-11β and H-24. H-26 with H-16β and H-18. H-27 with H-7, H-29a,b, AA_{OBz}', AA_{PABA}' and BB_{PABA}'. H-28 with H-22β, H-26 and H-30. H-29a,b with H-27 and H-30. H-30 with H-29a,b and AA_{OBz}'.

[c] Selected NOEs: H-6 with H-23 and H-24. H-7 with H-15α,β. H-11 with H-1α,β. H-23 with AA_{PABA}' and H-6. H-24 with H-3, H-6, H-23 and H-25. H-25 with H-11 and H-24. H-27 with H-29a,b, AA_{OBz}', AA_{PABA}' and BB_{PABA}'. H-28 with H-26. H-29a,b with H-27, H-30 and AA_{OBz}'. H-30 with H-29a,b, AA_{OBz}'.

extracts of various plants from the Cucurbitaceae family (Akihisa et al., 1997) might also be degradation products of compounds of the Δ^8-multiflorane-7β-ol type related to **1a**, as suggested by the easy formation of **2d** from **1b**.

The isolation of structurally unusual natural products from an edible plant highlights our limited knowledge on the occurrence of secondary metabolites in food, though evidence is mounting that certain secondary metabolites of fruits and vegetables might have effects on health beyond the obvious evidence of a classical deficiency syndrome (Pisha, & Pezzuto, 1994). The biochemical basis for the surprising profligacy by which certain plants from the genus *Cucurbita* incorporate PABA in secondary metabolites, the role of these compounds in the welfare and economy of the producer organism and the effect of their dietary uptake by humans are intriguing topics for further research.

3. Experimental

Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer model 237 spectropho-

tometer. HR-MS were taken on a MAT 95ST Finnigan MAT apparatus (70 eV, EI mode). ^{1}H- and ^{13}C NMR spectra were taken on Bruker AM 400 (400 and 100 MHz, respectively) and a Bruker DRX (500 and 125 MHz, respectively) instruments. ^{1}H- and ^{13}C-NMR chemical shifts refer to C*H*Cl$_3$ at 7.26 ppm and *C*DCl$_3$ at 77.0 ppm, respectively. Silica gel 60 (70–230 mesh, Merck) was used for open-column chromatography. A Waters Microporasil column (0.8 × 30 cm) was used for HPLC, with detection by a Waters differential refractometer 340.

3.1. Plant material

Seeds of pumpkin, zucchini and cucumber were purchased from Sementi Dotto, Mortigliano, UD, Italy. The seeds of buffalo gourd were obtained from fruits collected by AM around Fenix (Arizona, USA) in February 1998. Voucher specimens of all these seeds are kept at the Istituto Sperimentale per la Nutrizione delle Piante, Torino.

3.2. Isolation of the constituents

Isolation from the seeds of zucchini as representative (Varietà nano verde di Milano): dried and powdered seeds (500 g) were extracted with acetone at room temp. (1 × 2 l; 2 × 1 l). Removal of the solvent left a reddish oily residue (210 g) which was partitioned between hexane (1.0 l) and acetonitrile (1.0 l). After 16 h the two phases were separated and the lower acetonitrile phase was further washed with hexane (2 × 100 ml). Evaporation of the acetonitrile phase left a reddish residue (4.7 g) which was separated by column chromatography (60 ml silica gel, elution with mixtures of hexane–EtOAc). Elution with hexane–EtOAc 9:1 gave **2a** (65 mg after crystallisation from ether) as a white powder; elution with hexane–EtOAc 8:2 gave 342 mg **1a** as a green oil. Further purification was achieved by HPLC (microporasil column, hexane–EtOAc 8:2 as eluant) to give 210 mg of a crystalline powder. Attempts to remove the green colour from crude **1a** by C18 RP silica gel gave mainly the dehydrated product **2a**.

3.3. 3-O-p-Aminobenzoyl-29-O-benzoylmultiflora-8-ene-3α,7β,29-triol (*1a*)

Powder (Et_2O), m.p. 158–160°C; $[\alpha]_{25}^{D}$ −52 (pyridine, *c* 0.90); UV λ_{max}^{EtOH} nm (log ε): 289 (4.06); IR ν_{max}^{KBr} cm^{-1}: 3465, 3376, 1701, 1688, 1636, 1603, 1516, 1367, 1277, 1169, 1109, 714; CI-MS (isobutane) *m*/*z* (rel. int.): 682 $[C_{44}H_{59}NO_5+H]^+$ $[M+H]^+$ (100); HR-EIMS: 663.4290 $[M\text{-}H_2O]^+$ (2) (calculated for $C_{44}H_{57}NO_4$: 663.4288); ^{1}H NMR ($CDCl_3$): δ 8.04 (Bz-AA′), 7.79 (PABA-AA′), 7.61 (Bz-C), 7.53 (Bz-BB′), 6.67 (PABA-BB′), 4.82 (br s, H-3), 4.49 (br t, *J* = 6 Hz, H-7), 4.27 (d, *J* = 11 Hz, H-29a), 3.96 (d, *J* = 11 Hz, H-29b), 1.20 (s, H-26), 1.15, 1.13, 1.01 (s, H-23 + H-25 + H-28 + H-30), 0.97 (s, H-27).

3.4. 3-O-p-Aminobenzoyl-29-O-benzoylmultiflora-7,9(11)-diene-3α,29-diol (*2a*)

Powder (Et_2O), m.p. 204–206°C; $[\alpha]_{25}^{D}$ −130 ($CHCl_3$, *c* 0.90); UV λ_{max}^{EtOH} nm (log ε): 290 (4.06), 240 (sh); IR ν_{max}^{KBr} cm^{-1}: 3476, 3380, 1707, 1630, 1603, 1370, 1314, 1277, 1171, 1117; CI-MS (isobutane) *m*/*z* (rel. int.): 664 $[C_{44}H_{57}NO_4+H]^+$ $[M+H]^+$ (100). HR-EIMS: 663.4287 $[M]^+$ (1.5) (calculated for $C_{44}H_{57}NO_4$: 663.4288).

3.5. Acetylation of *1a*

To a soln. of **1a** (111 mg, 0.163 mmol) in pyridine (1.5 ml), an excess Ac_2O was added (1.5 ml). The reaction was stirred at room temp. for 24 h and then worked up by dilution with water and extraction with EtOAc. After washing with dil. HCl, $NaHCO_3$ and brine, the organic phase was dried (Na_2SO_4) and evaporated. The residue was purified by CC (ca. 5 ml silica gel, hexane–EtOAc 7:3 as eluant) to give 65 mg **1b** as a colourless powder, m.p. 161–162°C; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3374, 1707, 1599, 1526, 1370, 1279, 1173, 1113, 713; CI-MS (isobutane) *m*/*z* (rel. int.): 447 765 $[C_{48}H_{63}NO_7+H]^+$ $[M+H]^+$ (80).

3.6. Attempted silylation of *1a*

To a soln. of **1a** (70 mg, 0.103 mmol) in DMF (1.0 ml), imidazole (26 mg, 0.309 mmol, 3 mol. equiv.) and triethylsilyl chloride (65 ml, 58 mg, 3 mol. equiv.) were added. After stirring at room temp. overnight, the reaction was worked up by slowly pouring into a slurry of celite (ca. 1 g) in water (ca. 5 ml). The slurry was then filtered, and the cake washed with water to remove DMF and then with EtOAc to recover the product. After washing with brine and drying, the filtrate was evaporated and the residue purified by CC (ca. 5 g silica gel, hexane–EtOAc 7:3) to give 30 mg **2a** as a colourless powder.

3.7. Degradation of *1b* in $CDCl_3$

A sample of **1b** (ca. 10 mg) was dissolved in $CDCl_3$ and its ^{1}H NMR spectrum was taken at regular intervals. After 24 h, complete conversion to a ca. 10:1 mixture of **2b** and **2c** was observed. When the sample was exposed to the air, formation of **2d** was observed from **2b**. For the spectroscopic data of **2b** and **2d**, see Table 1Table 2. Diagnostical ^{1}H-NMR signals ($CDCl_3$) for **2c**: δ 4.84 (br s, H-3), 5.57 (br d, *J* = 5 Hz,

Table 2
^{13}C NMR data for **1a**, **1b**, **2a** (C_6D_6) and **2b** ($CDCl_3$) (125 MHz for **1a** and **1b**, 100 MHz for **2a** and **2b**)

Carbon	**1a**	**1b**[a]	**2a**[b]	**2b**
1	31.1 t	30.5 t	31.3 t	28.6 t
2	23.1 t	22.9 t	23.3 t	23.1 t
3	77.1 d	77.6 d	77.8 d	77.8 d
4	37.0 s	36.8 s	37.4 s	36.8 s
5	45.1 d	44.5 d	43.9 d	46.8 d
6	24.1 t	26.6 t	24.0 t	125.1 d
7	70.0 d	72.5 d	119.0 d	125.5 d
8	138.1 s	144.4 s	142.2 s	136.2 s
9	140.7 s	137.4 s	145.4 s	137.4 s
10	38.4 s	37.8 s	36.5 s	38.0 s
11	20.8 t	20.6 t	114.4 d	19.8 t
12	31.1 t	30.9 t	39.4 t	30.6 t
13	38.3 s	38.1 s	37.6 s	37.3 s
14	41.3 s	40.7 s	40.4 s	38.7 s
15	26.0 t	26.3 t	27.7 t	28.6 t
16	37.3 t	36.5 t	37.2 t	36.0 t
17	31.4 s	31.0 s	31.7 s	31.2 s
18	44.1 d	43.8 d	45.1 d	42.5 d
19	30.2 t	29.9 t	28.9 t	29.7 t
20	32.2 s	31.9 s	31.8 s	32.1 s
21	30.0 t	29.3 t	30.2 t	28.9 t
22	36.0 t	35.5 t	33.9 t	37.5 t
23	27.8 q	27.3 q	27.8 q	26.9 q
24	21.5 q	20.4 q	21.7 q	22.8 q
25	20.7 q	20.2 q	20.7 q	13.5 q
26	26.6 q	26.4 q	21.8 q	26.8 q
27	18.1 q	17.8 q	19.6 q	18.3 q
28	31.2 q	30.7 q	31.2 q	31.1 q
29	73.7 t	73.6 t	72.9 t	74.5 t
30	29.7 q	29.2 q	30.9 q	27.8 q
OBz	167.0 s 131.1 s 129.7 d 128.6 d 133.0 d	167.3 s 130.6 s 129.3 d 128.5 d 132.9 d	166.8 s 131.2 s 129.6 d 128.7 d 133.0 d	167.2 s 130.5 s 129.4 d 128.5 d 132.9 d
OPABA	165.7 s 120.7 s 131.8 d 114.0 d 151.8 s	164.6 s 126.3 s 130.8 d 118.6 d 143.2 s	165.6 s 120,7 s 131.8 d 113.8 d 151.3 s	165.3 s 126.3 s 130.7 d 118.8 d 142.0 s
NAc	- -	167.4 s 24.0 q	- -	168.5 s 24.7 q
OAc	–	169.5 s	–	–
	–	21.2 q	–	–

[a] Selected HMBC correlations: H-3/C=O_{PABA}; H-7/C=O_{OAc}; H-23/C-3; H-23/C-4; H-23/C-5; H-23/C-24. H-24/C-3; H-24/C-4; H-24/C-23; H-25/C-1; H-25/C-5; H-25/C-9; H-25/C-10; H-26/C-8; H-26/C-13; H-26/C-14; H-26/C-15. H-27/C-12; H-27/C-13; H-27/C-14; H-27/C-18. H-28/C-16; H-28/C-17; H-28/C-18; H-28/C-22; H-29a,b/C=O_{Obz}; H-30/C-19; H-30/C-20; H-30/C-21; H-30/C-29. AA′(OBz)/C=O_{OBz}; AA′(PABA)/C=O_{PABA}; NH/BB′(PABA); CH_3COOR/C=O_{OAc}. CH_3CONHR/C=O_{NAc}.

[b] Selected HMBC correlations: H-3/C=O_{PABA}; H-7/C-5; H-7/C-9; H-7/C-14. H-11/C-8; H-11/C-10; H-11/C-12; H-11/C-13; H-23/C-3; H-23/C-4; H-23/C-5; H-23/C-24; H-24/C-3; H-24/C-4; H-24/C-5; H-24/C-23; H-25/C-1; H-25/C-5; H-25/C-9; H-25/C-10; H-26/C-8; H-26/C-13; H-26/C-14; H-26/C-15; H-27/C-12; H-27/C-13; H-27/C-14; H-27/C-18; H-28/C-16; H-28/C-17; H-28/C-18; H-28/C-22; H-29a,b/C=O_{Obz}; H-29a,b/C-19; H-29a,b/C-20; H-29a,b/C-21; H-29a,b/C-30; H-30/C-19; H-30/C-20; H-30/C-21; H-30/C-29; AA′(OBz)/C=O_{OBz}; AA′(PABA)/C=O_{PABA}; NH/BB′(PABA).

H-7), 5.26 (br d, $J=5$ Hz, H-11), 0.89 (s, H-23), 0.94 (s, H-24), 0.99 (s, H-25), 1.04 (s, H-26), 0.99 (s, H-27), 1.11 (s, H-28), 4.20 (d, $J=11$ Hz, H-29a), 4.17 (d, $J=11$ Hz, H-29b), 1.14 (s, H-30). For **3**: δ 4.86 (br s, H-3), 6.52 (d, $J=9$ Hz, H-6), 6.84 (d, $J=9$ Hz, H-7), 5.49 (br d, $J=5$ Hz, H-11), 4.24 (d, $J=11$ Hz, H-29a), 4.04 (d, $J=11$ Hz, H-29b).

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References

Akihisa, T., Kimura, Y., Kasahara, Y., Kumaki, K., Thakur, S., & Tamura, T. (1997). *Phytochemistry*, *46*, 1261.

Akihisa, T., Kokke, W. C. M. C., Kimura, Y., & Tamura, T. (1993). *Journal of Organic Chemistry*, *58*, 1959.

Berge, J. M., & Roberts, S. M. (1979). *Synthesis*, 471.

Blakley, R. L. (1969). *The Biochemistry of Folic Acid and Related Pteridines*. North-Holland.

Bombardelli, E., & Morazzoni, P. (1997). *Fitoterapia*, *68*, 291.

Dictionary of Natural Products. Main Work and Supplements 1–4. Chapman and Hall.

Haslam, E. (1993). In *Shikimic Acid: Metabolism and Metabolites* (pp. 206–210). Wiley.

Pisha, E., & Pezzuto, J. M. (1994). Fruits and vegetables containing compounds that demonstrate pharmacological activity in humans. In H. Wagner, H. Hikino, & N. Farnsworth, Eds.), *Economic and Medicinal Plant Research (*, *Vol. 6* (pp. 189–223). Academic Press.

Pretsch, E., Seibl, J., Simon, W., & Clerc, T. (1989). In *Spectral Data for Structure Determination of Organic Compounds* (p. H255–H260). Springer.

Thompson, A. E. (1990). Arid-land industrial crops. In J. Janick, & J. E. Simon, Eds.), *Advances in New Crops (* (pp. 232–241). Portland, OR: Timber Press.

Woods, D. D. (1962). *General Microbiology*, *29*, 687.