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PATULETIN ACETYLRHAMNOSIDES FROM *KALANCHOE BRASILIENSIS* AS INHIBITORS OF HUMAN LYMPHOCYTE PROLIFERATIVE ACTIVITY

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ABSTRACT.—The fractionation of the juice of fresh stems and leaves of *Kalanchoe brasiliensis* was monitored by an assay measuring lymphocyte proliferative activity and allowed the isolation and identification of seven patuletin rhamnoside derivatives [**1**–**7**]. Three of them are novel, namely, patuletin 3-*O*-(4″-*O*-acetyl-α-L-rhamnopyranosyl)-7-*O*-(2‴-*O*-acetyl-α-L-rhamnopyranoside) [**1**], patuletin 3-*O*-α-L-rhamnopyranosyl-7-*O*-(2‴-*O*-acetyl-α-L-rhamnopyranoside) [**3**], and patuletin 3-*O*-(4″-*O*-acetyl-α-L-rhamnopyranosyl)-7-*O*-rhamnopyranoside [**4**], and four are known [**2**, **5**–**7**]. Their structures were determined by the analysis of ^{1}H-^{1}H and ^{1}H-^{13}C COSY nmr, ci, and fab mass spectra.

Of the seven flavonoids isolated, compounds **1**–**3** were shown to be potent inhibitors of lymphocyte proliferation.

Kalanchoe brasiliensis Camb. (Crassulaceae) is a Brazilian medicinal herb traditionally employed to treat tissue injuries, abcesses, enlarged ganglia, and inflammatory processes (1). Although *K. brasiliensis* is widely used in popular medicine in Brazil, its phytochemistry is still unknown. From the genus *Kalanchoe* (syn. *Bryophyllum*), which comprises about one hundred species, several classes of substances, such as bufadienolides (2,3), terpenoids (4), and flavonoids (5–7), have been reported previously.

In a previous study, the direct effect of *K. brasiliensis* juice on human lymphocyte activation in vitro was investigated in order to assess whether the alleged curative properties of this plant could be associated with a non-specific action on the immune system. It was shown that the crude lyophilized juice inhibited the lymphocyte proliferative response (8). Thus, a study monitored by lymphocyte proliferative assays (9) was undertaken to isolate and to identify the active compounds.

We report herein the isolation and the structure of three new patuletin acetyl di-*O*-rhamnosides from *K. brasiliensis* juice, kalambrosides A [**1**], B [**3**], and C [**4**], having immunomodulatory activity, along with the known **2** and **5** isolated earlier from *K. gracilis* (7), patuletin 3-*O*-rhamnoside [**6**] (10), and patuletin 3,7-di-*O*-rhamnoside [**7**] (5). Compounds **1**–**3** showed potent activity, whereas **4**–**7** had moderate to very low activity.

RESULTS AND DISCUSSION

Fresh stems and leaves of *K. brasiliensis* were expressed to yield a juice that was lyophilized and chromatographed on a reversed-phase Si gel column (RP-2) with a H_2O/MeOH gradient, yielding 10 fractions. The fraction that inhibited lymphocyte prolif-

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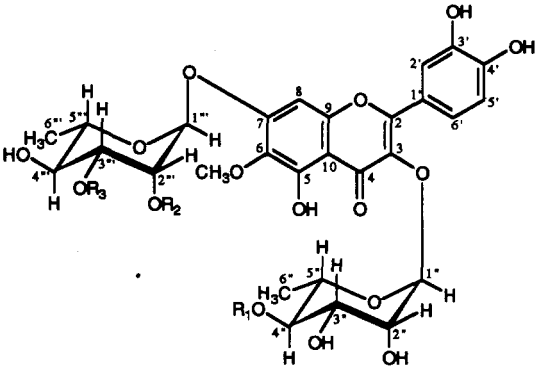

1 $R_1=R_2=Ac$, $R_3=H$
2 $R_1=R_3=Ac$, $R_2=H$
3 $R_2=Ac$, $R_1=R_3=H$
4 $R_1=Ac$, $R_2=R_3=H$
5 $R_3=Ac$, $R_1=R_2=H$
7 $R_1=R_2=R_3=H$

eration was further purified by a combination of reversed-phase Si gel (RP-8) and Sephadex LH-20 column chromatography, and afforded seven flavonoids [**1**–**7**].

The molecular formula for kalambroside A [**1**], $C_{32}H_{36}O_{18}$ was derived from the hr positive fab mass spectrum where the $[M+H]^+$ ion was observed at *m/z* 709.1965.

The ^{13}C-nmr spectrum of **1** displayed signals for 32 carbon atoms; eight of these occurred between δ 69 and 75, which, together with signals at δ 97.8 and 102.6, suggested the presence of two carbohydrate moieties. Analysis of the ^{1}H-nmr spectrum revealed the presence of two rhamnosyl residues, characterized by two doublet signals at δ 0.79 (J=6.2 Hz) and δ 1.27 (J=6.4 Hz) that corresponded to two -CH_3 groups. ^{1}H-^{1}H and ^{1}H-^{13}C COSY nmr spectra allowed the assignment of all the proton resonances of each rhamnosyl unit. The two doublets at δ 5.50 (J=1.6 Hz) and δ 5.61 (J=1.7 Hz) were assigned to the anomeric H-1″ and H-1‴, and the two singlet methyl signals at δ 2.04 and δ 2.15 to two acetyl groups (δ_C 21.1 and 20.9, respectively). The downfield signals at δ 5.29 (dd, J=3.6 and 1.7 Hz) and δ 4.82 (dd, J=9.5 and 9.5 Hz) resulted from the protons attached to the carbon-bearing acetoxyl group and were assigned to H-2‴ of a rhamnosyl unit, and to H-4″, respectively.

The remaining signals in the ^{13}C-nmr spectrum of **1** were one carbonyl at δ 179.6, eight oxygenated aryl carbon atoms between δ 131 and 160, two sp^2 quaternary carbon atoms at δ 122.7 and 107.5, and four protonated aromatic carbons between δ 100 and 123, in addition to a methoxyl signal at δ 62.4. The ^{1}H-nmr spectrum showed signals for four aromatic protons, a singlet at δ 6.63, and a three-proton system of an *o*, *p*-trisubstituted benzene ring at δ 7.38, 7.35, and 6.92, together with a methoxyl signal at δ 3.90 (Table 1). These ^{13}C- and ^{1}H-nmr signals were characteristic of the flavonol aglycone patuletin, whose protonated carbon assignment was based on its ^{1}H-^{13}C COSY data.

The unambiguous linkage of each acetylated rhamnosyl unit to the patuletin aglycone in **1** was established by analysis of the ^{1}H-^{13}C long-range COSY nmr spectrum. The long-range correlation observed between the carbon at δ 135.8 (C-3) and the anomeric proton at δ 5.50 (H-1″) indicated the attachment of the 4″-acetyl rhamnosyl unit to position 3 of the aglycone. On the other hand, the correlation observed between the carbon at δ 155.9 (C-7) and the anomeric proton at δ 5.61 (H-1‴) was indicative of a linkage of the 2‴-acetylrhamnosyl unit to position 7 of patuletin. The ^{1}H-^{13}C long-

TABLE 1. ^{1}H-Nmr Data for Patuletin 3,7-di-*O*-Rhamnoside [**7**], its Mono- and Diacetylated Derivatives [**1**–**5**], and Compound [**6**] (CD_3OD, 300 MHz).

Proton	Compound						
	1 δ (m,*J*)	**2** δ (m,*J*)	**3** δ (m,*J*)	**4** δ (m,*J*)	**5** δ (m,*J*)	**6** δ (m,*J*)	**7** δ (m,*J*)
Patuletin							
6-OCH_3	3.90 (s)	3.93 (s)	3.90 (s)	3.88 (s)	3.93 (s)	3.97 (s)	3.88 (s)
8	6.63 (s)	6.66 (s)	6.62 (s)	6.65 (s)	6.65 (s)	6.25 (s)	6.64 (s)
2′	7.38 (d 2.1)	7.39 (d 2.3)	7.42 (d 2.1)	7.38 (d 2.1)	7.40 (d 2.0)	7.40 (d 2.0)	7.42 (d 2.0)
5′	6.92 (d 8.3)	6.94 (d 8.2)	6.93 (d 8.1)	6.93 (d 8.4)	6.93 (d 8.5)	6.93 (d 8.3)	6.93 (d 8.2)
6′	7.35 (dd 8.3,2.1)	7.35 (dd 8.2,2.3)	7.39 (dd 8.1,2.1)	7.33 (dd 8.4,2.1)	7.39 (dd 8.5,2.0)	7.36 (dd 8.3,2.0)	7.39 (dd 8.2,2.0)
3-*O*-Rha							
1″	5.50 (d 1.6)	5.51 (br s)	5.37 (d 1.7)	5.50 (d 1.6)	5.37 (br s)	5.35 (br s)	5.37 (br s)
2″	4.22 (dd 3.3,1.6)	4.23 (br s)	4.23 (dd 3.2,1.7)	4.22 (dd 3.4,1.6)	4.24 (br s)	4.23 (br s)	4.24 (br s)
3″	3.90 (dd 9.5,3.3)	3.92 (dd 9.0,3.0)	3.77 (dd 9.2,3.2)	3.90 (dd 9.9,3.4)	3.78 (dd 8.5,3.0)	3.76 (dd 9.0,3.0)	3.77 (dd 9.5,3.3)
4″	4.82 (dd 9.5,9.5)	4.86 (m)	3.34 (dd 9.2,9.2)	4.82 (dd 9.9,9.9)	3.33 (dd 8.5,8.5)	3.34 (dd 9.0,9.0)	3.35 (dd 9.5,9.5)
5″	3.34 (dq 9.5,6.2)	3.34 (m)	3.45 (dq 9.2,6.1)	3.34 (dq 9.9,6.3)	3.45 (m)	3.44 (m)	3.45 (m)
6″-CH_3	0.79 (d 6.2)	0.80 (d 6.1)	0.95 (d 6.1)	0.79 (d 6.3)	0.96 (d 6.1)	0.95 (d 6.2)	0.95 (d 6.1)
4″-OAc	2.04 (s)	2.05 (s)	—	2.04 (s)	—	—	—
7-*O*-Rha							
1‴	5.61 (d 1.7)	5.59 (br s)	5.61 (d 1.7)	5.57 (d 1.6)	5.58 (br s)	—	5.57 (br s)
2‴	5.29 (dd 3.6,1.7)	4.27 (br s)	5.29 (dd 3.6,1.7)	4.10 (dd 3.5,1.6)	4.27 (br s)	—	4.11 (br s)
3‴	4.08 (dd 9.6,3.6)	5.20 (dd 9.5,3.5)	4.08 (dd 9.4,3.6)	3.88 (dd 9.5,3.5)	5.20 (dd 9.3,3.2)	—	3.90 (dd 9.5,3.5)
4‴	3.46 (dd 9.6,9.6)	3.71 (dd 9.5,9.5)	3.47 (dd 9.4,9.4)	3.50 (dd 9.5,9.5)	3.72 (dd 9.3,9.3)	—	3.50 (dd 9.5,9.5)
5‴	3.73 (dq 9.6,6.4)	3.75 (m)	3.73 (dq 9.4,6.1)	3.65 (dq 9.5,6.1)	3.65 (m)	—	3.66 (dq 9.5,6.1)
6‴-CH_3	1.27 (d 6.4)	1.29 (d 5.5)	1.27 (d 6.1)	1.26 (d 6.1)	1.28 (d 5.6)	—	1.26 (d 6.1)
2‴-OAc	2.15 (s)	—	2.15 (s)	—	—	—	—
3‴O-Ac	—	2.16 (s)	—	—	2.16 (s)	—	—

range COSY spectrum also showed correlations between the methoxyl protons at δ 3.90 and the carbon at δ 131.0 (C-6), as well as the expected correlations between H-8 and C-10, C-9, C-7, C-6; between H-6′ and C-2, C-2′ and C-4′; and between H-5′ and both C-3′ and C-6′. The characteristic ion corresponding to the protonated patuletin aglycone is observed in the cims at *m/z* 333. The presence of acetylrhamnosyl groups is confirmed by ions at *m/z* 206 ($C_8H_{14}O_6$) and 189.

The structure of kalambroside A, a new patuletin glycoside, was therefore determined as patuletin 3-*O*-(4″-*O*-acetyl-α-L-rhamnopyranosyl)-7-*O*-(2‴-*O*-acetyl-α-L-rhamnopyranoside) [**1**].

Compound **2** had two monoacetylated rhamnosyl units and the signal pattern of the patuletin aglycone. Its complete structural assignment was achieved on the basis of ^{1}H-^{13}C-nmr (direct and long-range) experiments, allowing us to link the two acetoxyl groups to the C-4″ position and C-3‴ of the two rhamnosyl residues at C-3 and C-7 of the patuletin aglycone, respectively (Tables 1 and 2). Compound **2** was thus identified as patuletin 3-*O*-(4″-*O*-acetyl-α-L-rhamnopyranosyl)-7-*O*-(3‴-*O*-acetyl-α-L-rhamnopyranoside), previously isolated from *K. gracilis* (7).

The positive hrfabms spectrum for kalambroside B [**3**] displayed a $[M+H]^+$ ion at *m/z* 667.1877 corresponding to $C_{30}H_{34}O_{17}$ as molecular formula, with one acetyl group fewer than **1** and **2**. The ^{1}H- and ^{13}C-nmr spectral data of **3** were similar to those of **1**. They depicted the presence of the patuletin aglycone and two rhamnosyl units (Tables 1 and 2). One rhamnosyl unit was acetylated at position C-2‴, as demonstrated by the deshielded signal of H-2‴ at δ 5.29 (dd, J=3.6 and 1.7 Hz). The connection pattern of the two rhamnosyl residues on the aglycone was established by long-range ^{1}H-^{13}C COSY experiments.

The non-acetylated rhamnosyl unit was linked to C-3 of patuletin aglycone in **3** as judged from the correlation observed between the carbon at δ 136.5 (C-3) and the anomeric proton at δ 5.37 (H-1″). The position of attachment of the 2‴-acetylrhamnosyl unit resulted from the correlation observed between the carbon at δ 155.8 (C-7) and the anomeric proton at δ 5.61 (H-1‴). The fabms showed the characteristic fragments for patuletin aglycone (*m/z* 333) and a fragment resulting from the loss of the 3-*O*-rhamnosyl unit (*m/z* 521). Kalambroside B was thus patuletin 3-*O*-(α-L-rhamnopyranosyl)-7-*O*-(2‴-*O*-acetyl-α-L-rhamnopyranoside) [**3**], a new patuletin acetylrhamnopyranoside.

Kalambroside C [**4**] had a molecular formula of $C_{30}H_{34}O_{17}$ as determined from the positive hrfabms displaying a $[M+H]^+$ ion at *m/z* 667.1875. Analysis of ^{1}H-, ^{1}H-^{1}H, and ^{1}H-^{13}C COSY nmr spectra revealed that **4** was a monoacetyl dirhamnoside of patuletin (Tables 1 and 2). The low-field signal at δ 4.82 (dd) assigned to the rhamnosyl proton H-4″, and the signal at δ 2.04 indicated acetylation of the hydroxyl group at C-4″. Methyl doublets of rhamnosyl moieties were observed at δ 0.79 (J=6.3 Hz) and δ 1.26 (J=6.1 Hz), as for **1**, and suggested the same 3-*O*- and 7-*O*- substitution pattern. Anomeric proton signals were at δ 5.50 (J=1.6 Hz; H-1″) and δ 5.57 (J=1.6 Hz; H-1‴). The linkage of each rhamnosyl unit was determined based on long-range ^{1}H-^{13}C nmr experiments. The structure of kalambroside C [**4**] was identified as patuletin 3-*O*-(4″-*O*-acetyl-α-L-rhamnopyranosyl)-7-*O*-rhamnopyranoside, a new flavonoid acetyl glycoside.

Compound **5** had a ci mass spectrum which depicted the $[M+H]^+$ ion at *m/z* 667, and nmr spectra characteristic of a monoacetyldirhamnosyl-patuletin. Its ^{1}H-nmr spectrum showed only one acetyl group linked at C-3‴ of the acetylated rhamnosyl residue and compound **5** was thus identified as patuletin 3-*O*-(α-L-rhamnopyranosyl)-7-*O*-(3‴-*O*-acetyl-α-L-rhamnopyranoside), previously isolated from *K. gracilis* (7). Compounds **6** and **7** were similarly identified as patuletin 3-*O*-α-L-rhamnopyranoside (9) and patuletin 3-*O*-α-L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside (5).

TABLE 2. ^{13}C-Nmr Data for Patuletin 3,7-di-*O*-Rhamnoside [**7**], its Mono and Diacetylated Derivatives [**1–5**] and Compound [**6**] (CD_3OD, 75 MHz).

Carbon	Compound						
	1 δ	**2** δ	**3** δ	**4** δ	**5** δ	**6** δ	**7** δ
Patuletin							
2	159.9	159.7	159.6	159.9	159.6	159.1	159.6
3	135.8	135.7	136.5	135.8	136.4	136.2	136.5
4	179.6	179.4	179.8	179.6	179.8	179.7	179.8
5	150.1	149.8	150.0	150.0	150.0	150.3	150.0
6	131.0	130.8	131.2	130.8	131.0	129.0	130.8
7	155.9	155.9	155.8	156.2	156.1	158.3	156.2
8	100.0	99.7	99.9	99.8	99.8	100.0	99.7
9	157.6	157.4	157.6	157.6	157.7	158.0	157.6
10	107.5	107.2	107.5	107.2	107.4	105.8	107.2
1′	122.7	122.6	122.8	122.7	122.8	123.0	122.8
2′	116.9	116.9	116.9	116.9	116.9	116.9	117.0
3′	146.5	146.4	146.3	146.5	146.4	146.3	146.3
4′	150.0	149.8	149.9	149.9	150.0	149.9	149.9
5′	116.3	116.2	116.4	116.3	116.4	116.5	116.4
6′	123.0	123.1	123.1	123.0	123.2	123.0	123.2
6-OCH_3	62.4	62.3	62.4	62.3	62.4	62.0	62.3
3-*O*-Rha							
1″	102.6	102.5	103.5	102.6	103.5	103.5	103.6
2″	71.7	71.6	71.9	71.7	71.9	71.9	71.9
3″	70.0	69.9	72.0	70.0	72.2	72.1	72.0
4″	75.0	74.9	73.2	74.9	73.2	73.2	73.2
5″	69.6	69.4	72.0	69.6	72.1	72.0	72.0
6″	17.5	17.5	17.6	17.5	17.6	17.6	17.6
4″-OAc (CH_3)	21.1	21.1	—	21.0	—	—	—
4″-OAc (CO)	172.6	172.7	—	172.6	—	—	—
7-*O*-Rha							
1‴	97.8	100.0	97.8	100.3	100.2	—	100.3
2‴	73.3	69.4	73.5	71.7	69.5	—	71.7
3‴	70.4	75.2	70.4	72.1	75.3	—	72.1
4‴	73.7	70.7	73.7	73.5	70.8	—	73.5
5‴	71.4	71.4	71.4	71.4	71.5	—	71.4
6‴	18.0	18.0	18.0	18.0	18.0	—	18.1
2‴-OAc (CH_3)	20.9	—	20.9	—	—	—	—
2‴-OAc (CO)	172.3	—	172.3	—	—	—	—
3‴-OAc (CH_3)	—	21.0	—	—	21.1	—	—
3‴-OAc (CO)	—	172.6	—	—	172.7	—	—

The examination of ^{1}H-nmr data (Table 1) for these acetyl flavonoids allowed us to observe that for the 3-*O*-linked rhamnosyl unit, 4″-*O*-acetylation induced downfield shifts of the anomeric proton H-1″ (Δδ +0.13 ppm) and H-3″ (Δδ +0.13 ppm) signals, but upfield shifts of H-5″ (Δδ −0.11 ppm) and H-6″ (Δδ −0.16 ppm). *O*-Acetylation of the 7-*O*-rhamnosyl group at position 2‴ normally deshielded the vicinal H-3‴ by Δδ 0.2 ppm, whereas acetylation at position 3‴ induced a deshielding of both H-2‴ and H-4‴ by Δδ 0.2 ppm and had no significant effect on the anomeric proton H-1‴.

The data in Table 2 indicate that acetylation of the equatorial hydroxyl group at C-4″ induces a shift of the C-4″ signal to lower field (+1.7 ppm) and shifts of the C-1″, C-3″, and C-5″ signals to higher field (−1.0, −2.0 and −2.5 ppm, respectively). Similar shifts were observed by acetylation of the equatorial C-3‴ hydroxyl group, leading to the

following shift variations: C-3‴ (+3.0 ppm), C-2‴ (−2.3 ppm), and C-4‴ (−2.8 ppm). Acetylation of the axial C-2‴ hydroxyl group induced a shift of the C-2‴ signal (+1.7 ppm), the C-1‴ signal (−2.6 ppm), and the C-3‴ signal (−1.8 ppm).

The seven flavonoids isolated from *K. brasiliensis* were tested in a lymphoproliferative assay. Table 3 shows that lymphocyte proliferation was inhibited by 50% at concentrations of 0.25 μg/ml of **2** or 0.5 μg/ml of kalambroside A [**1**]. These concentrations are, respectively, 80- and 40-fold less than those required when the crude juice was added to the culture medium. The table also shows that diacetylated flavonoids [**2**] and kalambroside A [**1**] were more potent in blocking proliferation than the monoacetylated derivatives kalambrosides B [**3**], C [**4**], and compound **5**. The comparative analysis of the data (Table 3) allows one to suppose that the spatial position of the acetyl group on the rhamnosyl unit plays an important role in the lymphoproliferative inhibitory activity of these flavonoids. The patuletin monorhamnoside [**6**] and the patuletin non-acetylated dirhamnoside [**7**] were devoid of activity.

The mechanism of action of these acetyldirhamnosyl flavonoids is under investigation.

TABLE 3. Suppression of PHA-Induced Human Lymphocyte Proliferation by the Crude Lyophilized Juice of *K. brasiliensis* and Compounds [**1–5**] Expressed as IC_{50}.

Extract/compound added[a]	IC_{50} (μg/ml)[b]
Crude lyophilized juice ...	20.0
1	0.5
2	0.25
3	1.0
4	>10.0
5	10.0

[a]Crude juice or purified compounds were added at the beginning of the culture period and experiments were done in triplicate.

[b]Concentration to inhibit 50% of the proliferative response of lymphocytes to 5 μg/ml PHA.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Reversed-phase cc was carried out on silanized Si 60 gel (70–230 mesh) Merck, on Lichroprep RP8 (40–63) Merck and on Sephadex LH-20 (Pharmacia) using a H_2O/MeOH gradient with increasing proportions of MeOH. Elutions were monitored by tlc (silica 60 F_{254}, Merck) using BuOH-AcOH-H_2O (8:1:1) and EtOAc-Me_2CO-HOAc-H_2O (30:3:1:1) as solvent systems. Chromatograms were visualized under uv (254 and 365 nm) and by spraying an ethanolic vanillin/H_2SO_4 solution followed by heating. The $[\alpha]_D$ values were determined with a Perkin-Elmer Model 243 B polarimeter and ir spectra were registered on a Nicolet Model 205 Ft-ir spectrometer. Nmr spectra were recorded on a Bruker AC 300 spectrometer (^1H, 300 MHz; ^{13}C, 75 MHz; CD_3OD), with the CHD_2OD signal taken as internal reference (δ 3.313). Long-range ^1H-^{13}C COSY nmr spectra were obtained with $J=7$ Hz. Cims and positive fabms were obtained on a NERMAG R 10-10 and on a ZAB-HF mass spectrometer, respectively.

PLANT MATERIAL.—*Kalanchoe brasiliensis* Camb. was collected in the spring of 1992 in Rio de Janeiro, Brazil. A voucher specimen is on deposit in the herbarium of the Botanical Garden of Rio de Janeiro (304 627 Camb.). Fresh stems and leaves were expressed together in a food processor. The green juice obtained was clarified by filtration on Whatman No. 1 paper and lyophilized, affording a light-yellow powdered material.

EXTRACTION AND ISOLATION.—The lyophilized juice (36 g from 1800 g of fresh plant) was dissolved

in distilled H_2O (140 ml) and chromatographed on a reversed-phase column (RP-2 silanized Si gel). The elution with a gradient H_2O/MeOH gave 10 fractions (F1–F10). The flavonoid-enriched fraction (F9, 1.0 g) showed significant inhibition of lymphocyte proliferative activity. It was purified by reversed-phase (Lichroprep RP-8) cc eluted with a H_2O-MeOH gradient from 1:1 to 0:1 and 15 fractions (f1 to f15) were obtained. Fraction f6 yielded pure **7** (170 mg); f7 (97 mg) was chromatographed on Sephadex LH-20 (H_2O-MeOH, 1:1) and yielded **5** (22 mg); f8 (386 mg) was chromatographed on reversed-phase (Lichroprep RP-8, H_2O-MeOH, 1:1) yielding in the last fractions, pure compound **3** (39 mg), and in the first fractions, a mixture of **4** and **6** which were further separated by chromatography on Sephadex LH-20 (H_2O-MeOH, 1:1) to afford pure **4** (70 mg) and **6** (21 mg); fractions f10 and f12 contained pure **2** (151 mg) and **1** (132 mg), respectively. The chromatographic fractionations were monitored by the lymphocyte proliferative bioassay; the in vitro peripheral blood mononuclear cell proliferation assays were carried out according to procedures described by Moraes *et al.* (9).

Kalambroside A [*patuletin 3-O-(4″-O-acetyl-α-L-rhamnopyranosyl)-7-O-(2‴-O-acetyl-α-L-rhamnopyranoside*] [**1**].—$C_{32}H_{36}O_{18}$ (132 mg); $[\alpha]_D^{21}$ −128° (*c*=0.12, MeOH); ir ν max (neat) 3424, 3408, 2930, 1729, 1650, 1601, 1499, 1448, 1370, 1304, 1255, 1197, 1165, 1123, 1079, 1032, 963, 697 cm^{-1}; uv λ max (MeOH) 356 (log ε 4.24), 268 (4.44), 263 (4.45) nm; ^1H and ^{13}C nmr (CD_3OD), see Tables 1 and 2; positive fabms *m/z* $[M+H]^+$ 709 (32), 521 (27), 333 (71), 303 (9), 301 (12) and 189 (100); cims *m/z* 563 (8), 547 (5), 521 (9), 505 (8), 463 (2), 333 (40), 317 (32), 273 (2), 231 (9), 206 (2), 189 (48), 171 (20), 146 (7) and 129 (100); hrfabms $[M+H]^+$ 709.1965 (calcd for $C_{32}H_{37}O_{18}$, 709.1980).

Patuletin 3-O-(4″-O-acetyl-α-L-rhamnopyranosyl)-7-O-(3‴-O-acetyl-α-L-rhamnopyranoside) [**2**].—$C_{32}H_{36}O_{18}$ (151 mg); ^1H and ^{13}C nmr (CD_3OD), see Tables 1 and 2; cims *m/z* $[M+H]^+$ 709 (1), 563 (20), 547 (2), 521 (36), 505 (15), 479 (15), 333 (100), 317 (44), 287 (1), 231 (16), 206 (9), 189 (77), 171 (21), 146 (6), and 129 (61).

Kalambroside B [*patuletin 3-O-α-L-rhamnopyranosyl-7-O-(2‴-O-acetyl-α-L-rhamnopyranoside)*] [**3**].—$C_{30}H_{34}O_{17}$ (39 mg); $[\alpha]_D^{21}$ −91° (*c*=0.15, MeOH); ir ν max (neat) 3417, 3408, 2936, 1726, 1651, 1600, 1502, 1446, 1365, 1303, 1258, 1198, 1168, 1123, 1080, 1032, 999, 962 cm^{-1}; uv λ max (MeOH) 355 (log ε 4.30), 260 (4.49) nm; ^1H and ^{13}C nmr (CD_3OD), see Tables 1 and 2; positive fabms *m/z* $[M+H]^+$ 667 (20), 521 (27), 479 (7), 333 (100), 303 (17), 301 (9), and 189 (55); cims *m/z* 563 (1), 505 (1), 479 (4), 346 (1), 333 (87), 317 (62), 273 (1), 189 (8), 171 (10), 147 (8), 129 (100), and 113 (8); hrfabms $[M+H]^+$ 667.1877 (calcd for $C_{30}H_{35}O_{17}$ 667.1874).

Kalambroside C [*patuletin 3-O-(4″-O-α-L-rhamnopyranosyl)-7-O-α-L-rhamnopyranoside*] [**4**].—$C_{30}H_{34}O_{17}$ (170 mg); $[\alpha]_D^{21}$ −168° (*c*=0.12, MeOH); ir ν max (neat) 3428, 3409, 3166, 3088, 2932, 1729, 1650, 1601, 1500, 1447, 1368, 1305, 1263, 1197, 1119, 1071, 1030, 1001, 961, 694 cm^{-1}; uv λ max (MeOH) 357 (log ε 4.35), 260 (4.56) nm; ^1H and ^{13}C nmr (CD_3OD), see Tables 1 and 2; positive fabms *m/z* $[M+H]^+$ 667 (22), 521 (3), 479 (11), 333 (47), 305 (5), 301 (3), and 189 (27); cims *m/z* $[M+H]^+$ 667 (0.5), 607 (0.5), 563 (9), 535 (1), 521 (34), 503 (0.5), 479 (43), 461 (1), 443 (0.5), 346 (1), 333 (100), 317 (34), 289 (2), 260 (1), 206 (3), 189 (33), 171 (9), 147 (9), 129 (77), 99 (5) and 85 (17); hrfabms $[M+H]^+$ 667.1875 (calcd for $C_{30}H_{35}O_{17}$ 667.1874).

Patuletin 3-O-α-L-rhamnopyranosyl-7-O-(3‴-O-acetyl-α-L-rhamnopyranoside) [**5**].—$C_{30}H_{34}O_{17}$ (22 mg); ^1H and ^{13}C nmr (CD_3OD), see Tables 1 and 2; cims *m/z* 521 (4), 479 (7), 405 (1), 347 (2), 333 (59), 317 (26), 239 (2), 189 (9), 129 (100).

Patuletin 3-O-α-L-rhamnopyranoside [**6**].—$C_{22}H_{22}O_{12}$ (21 mg); ^1H and ^{13}C nmr (CD_3OD), see Tables 1 and 2; cims *m/z* $[M+H]^+$ 479 (5), 347 (2), 333 (85), 317 (41), 301 (2), 164 (5), and 129 (100).

Patuletin 3-O-α-L-rhamnopyranosyl-7-O-α-L-rhamnopyranoside [**7**].—$C_{28}H_{32}O_{16}$ (170 mg); ^1H and ^{13}C nmr (CD_3OD), see Tables 1 and 2; cims *m/z* 493 (1), 471 (9), 347 (8), 333 (66), 317 (35), 301 (2), 237 (1), 219 (1), 164 (11), 147 (11), 129 (100), 120 (17), and 85 (9).

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