Composite Constituents: Forty-Two Triterpenoids Including Eight Novel Compounds Isolated from *Picris hieracioides* subsp. *japonica*

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Forty-two triterpenoids including eight novel compounds, gammacer-16-en-3β-yl acetate (1), gammacer-16-en-3β-ol (2), gammacer-16-en-3α-ol (3), gammacer-16-en-3-one (4), pichierenyl acetate (5), pichierenone (6), isopichierenyl acetate (7) and isopichierenol (8), were isolated from the fresh roots of *Picris hieracioides* subsp. *japonica*, Compositae, and their structures were elucidated by means of spectroscopic analysis, and chemical correlations. Fifteen compounds were also obtained from the fresh aerial parts.

Key words *Picris hieracioides* subsp. *japonica*; Compositae; triterpenoid; gammacer-16-en-3β-yl acetate; pichierenyl acetate

Picris hieracioides LINNÉ is a common composite weed, distributed from Europe to Asia, of which subsp. *japonica* (THUNB) KRYLOV. (kôzorina in Japanese) is found widely at the roadside by fields and on hills in Japan. This species characteristically contains abundant milky liquid, in which many triterpenoid components are found. We have isolated eight novel triterpenoids, gammacer-16-en-3β-yl acetate (**1**),[1] gammacer-16-en-3β-ol (**2**),[1] gammacer-16-en-3α-ol (**3**),[1] gammacer-16-en-3-one (**4**), pichierenyl acetate (**5**),[2] pichierenone (**6**), isopichierenyl acetate (**7**)[2] and isopichierenol (**8**), together with thirty-four known triterpenoids **9**—**42** from the fresh roots (Chart 1). This paper deals with the isolation of compounds belonging to the triterpenoid acetate, ketone and alcohol classes, and the structure elucidation of compounds **1**—**8** by means of extensive spectroscopic analysis, and chemical correlation. Fifteen compounds, **10**—**17**, **32**—**34**, **36**, and **38**—**40**, were also obtained from the fresh aerial parts of this plant.

Results and Discussion

The fresh roots of *P. hieracioides* subsp. *japonica* were extracted with hexane, and the extracts were separated by various kinds of chromatography (see Experimental) to give compounds **1**—**42**, which are presented in Table 1 with their physical constants and yields. The mixture of triterpenoid alcohols **31**—**42** were acetylated and the products were identified as the corresponding acetates.

Compound **1** was obtained as colorless plates, and the high-resolution MS (HR-MS) of **1** indicated M^+ at *m/z* 468.3948 (Calcd 468.3967) suggesting the molecular formula to be $C_{32}H_{52}O_2$. The IR absorption of **1** suggested the presence of an acetoxyl group. The low-resolution MS (LR-MS) of **1** showed the base peak at *m/z* 189 (a) and other major fragment ions at *m/z* (relative intensity) 204 (34, b), 203 (33, c), 187 (97, d), 150 (38, e) (Chart 2). This fragment pattern has never been observed in known triterpenoid ring systems,[3] but the fragment ion at *m/z* 189 (base peak) indicates that the A, B and C rings of **1** could be the same as those of tetrahymanyl acetate (**43**).[3] A new carbon system having a Δ^{16} double bond was suggested for **1**, because the latter two fragment ions (e and f) were considered to have been generated by an allylic cleavage in ring D. The ^{1}H-NMR spectrum of **1** indicated the presence of eight tertiary methyl groups, a trisubstituted double bond [δ 5.441 (dd, J=3.0, 4.9 Hz)] and an acetoxy methine [δ 4.488 (dd, J=5.9, 10.6 Hz)]. The methyl protons at positions 23, 24, 25, 26 and 27 of **1** were observed at almost the same field as those of **43**, but those at positions 28, 29 and 30 appeared at lower fields (Table 2). In the ^{13}C-NMR spectrum (Table 3), the signals of **1** were coincident with those of **43** except for the double bond and adjacent carbons. Assignments of ^{1}H- and ^{13}C-NMR spectra of the compounds shown in Tables 2 and 3 were confirmed by proton–proton and ^{13}C-proton correlated spectroscopy (^{1}H–^{1}H and ^{13}C–^{1}H COSY), heteronuclear single quantum coherence spectroscopy (HSQC), ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectrum and distortionless enhancement by polarization transfer (DEPT) spectrum methods. The relative configuration of **1** was established by nuclear Overhauser effect spectroscopy (NOESY). That is, NOE interactions were observed between methyl and methyl or methine groups on the α-side of the molecule (H-23–H-5–H-9–H-27–H-28–H-29), and on the β-side (H-26–H-13). The above results strongly suggested that the structure of **1** is gammacer-16-en-3β-yl acetate.

Compound **2** was obtained as colorless plates and the HR-MS of **2** indicated the molecular formula to be $C_{30}H_{50}O$ (*m/z* 426.3873). The IR spectrum of **2** showed the presence of a hydroxyl group. Compound **2** was identified by comparison with the alcohol, gammacer-16-en-3β-ol, derived from **1**. Compound **3** was obtained as colorless plates, and the HR-MS of **3** indicated the molecular formula to be $C_{30}H_{50}O$ (*m/z* 426.3881). The IR spectrum of **3** indicated the presence of a hydroxyl group, and the configuration was determined to be 3α on the basis of the coupling [δ 3.390 (dd, J=2.8, 2.8 Hz)] observed for H-3 in the ^{1}H-NMR spectrum. Compound **4** was obtained as colorless plates, and the HR-MS of **4** indicated the molecular formula to be $C_{30}H_{48}O$ (*m/z* 424.3699). The IR spectrum of **4** indicated the presence of a carbonyl group. Compound **4** was obtained from **2** and **3** by CrO_3–pyridine complex oxidation, and therefore, the structures of **3** and **4** were established as gammacer-16-en-3α-ol and gammacer-16-en-3-one, respectively.

Compounds **5**, named pichierenyl acetate,[2] and **7**, named isopichierenyl acetate,[2] were obtained as colorless

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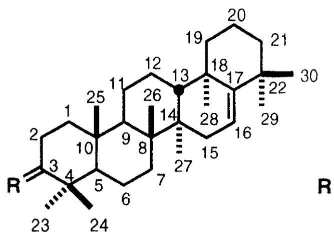

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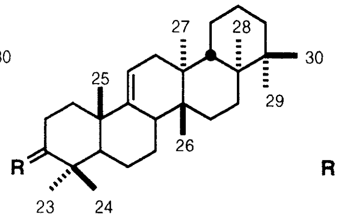
2 R=αH, βOH
3 R=αOH, βH
4 R=O



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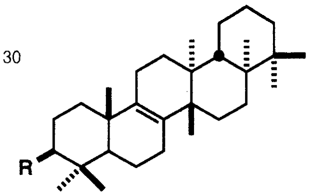
6 R=O
29 R=αH, βOH



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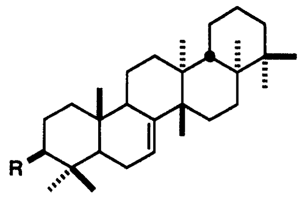

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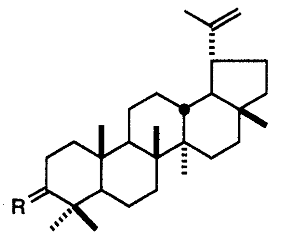
30 R=OH



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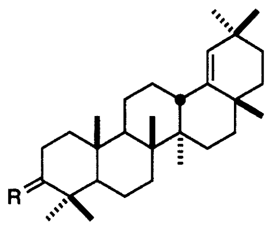
20 R=O
32 R=αH, βOH



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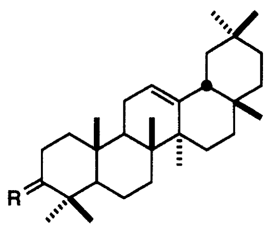
21 R=O
33 R=αH, βOH



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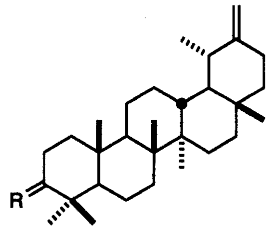
34 R=αH, βOH



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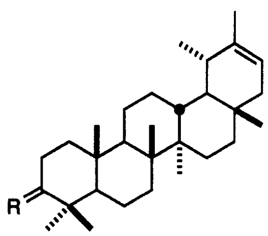
24 R=O
36 R=αH, βOH



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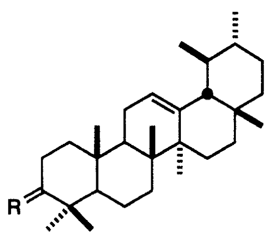
25 R=O
37 R=αH, βOH



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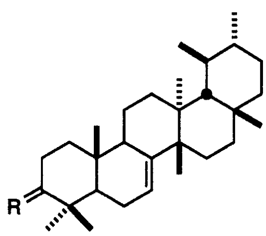
26 R=O
38 R=αH, βOH



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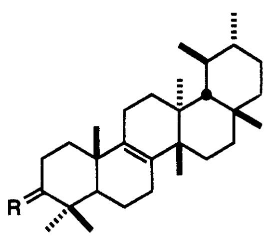
39 R=αH, βOH



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28 R=O
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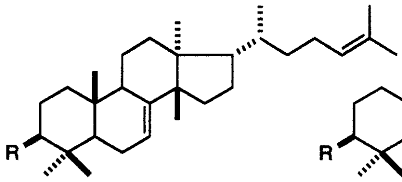

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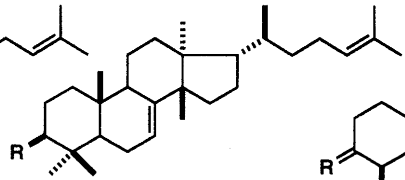
41 R=OH



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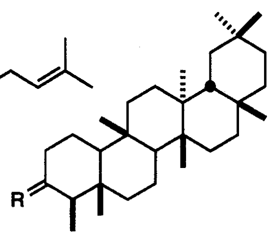
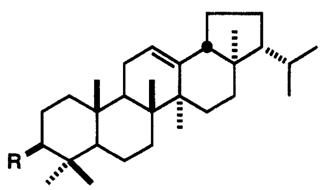
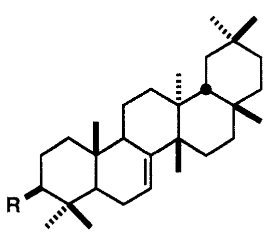
42 R=OH



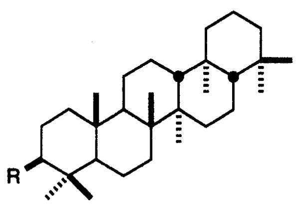
23 R=O



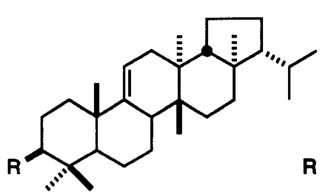
31 R=OH



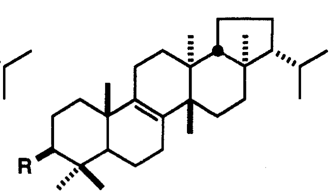
35 R=OH



43 R=OAc



44 R=OAc



45 R=OAc

Chart 1

Table 1. Triterpenoids Isolated from *Picris hieracioides* subsp. *japonica*

	mp (°C)	$[\alpha]_D$ (°)	Yield[a] (%) Roots	Ref.		mp (°C)	$[\alpha]_D$ (°)	Yield[a] (%) Roots	Ref.
Gammacer-16-en-3β-yl acetate (**1**)	287—288	+36.0	0.0138	1	β-Amyrenone (**22**)	166—167		0.0007	5
Gammacer-16-en-3β-ol (**2**)	269—270	+36.6	0.0010	1	Friedelin (**23**)			0.00003	6
Gammacer-16-en-3α-ol (**3**)	277—277.5	+9.9	0.0692	1	Taraxasterone (**24**)	184—185		0.0016	6
Gammacer-16-en-3-one (**4**)	271—272	+58.3	0.0004		ψ-Taraxasterone (**25**)	168—169		0.0006	6
Pichierenyl acetate (**5**)	272.5—273.5	−31.2	0.0009	2	α-Amyrenone (**26**)	120—121		0.0011	6
Pichierenone (**6**)	220—221	−71.0	0.0002		Bauerenone (**27**)			0.000009	6
Isopichierenyl acetate (**7**)	248.5—249.5	−2.6	0.0002	2	Isobauerenone (**28**)	182—183		0.0003	6
Isopichierenol (**8**)	239—240	−9.5	0.00005		Pichierenol (**29**)	248.5—249.5	−44.3	0.0003	9
Swertenyl acetate (**9**)	242—242.5	−34.4	0.0005	4	Swertenol (**30**)	238—239	−51.0	0.00003	4
Lupenyl acetate (**10**)	221—221.5		0.0294	5	Neomotiol (**31**)			0.00002	11
Germanicyl acetate (**11**)	281—283		0.0169	5	Lupeol (**32**)			0.0241	5
β-Amyrin acetate (**12**)	246—247		0.0472	5	Germanicol (**33**)			0.0003	5
Taraxasteryl acetate (**13**)	250—251		0.1286	5	β-Amyrin (**34**)			0.0089	5
ψ-Taraxasteryl acetate (**14**)	238—239		0.0303	5	Multiflorenol (**35**)			0.0014	10
α-Amyrin acetate (**15**)	229—230		0.0885	5	Taraxasterol (**36**)			0.0082	5
Bauerenyl acetate (**16**)	296—297		0.0210	5	ψ-Taraxasterol (**37**)			0.0041	5
Isobauerenyl acetate (**17**)	229—230		0.0112	6	α-Amyrin (**38**)			0.0025	5
Butyrospermyl acetate (**18**)	141—142	+15.1	0.0002	7	Bauerenol (**39**)			0.0127	5
Tirucalla-7,21-dien-3β-yl acetate (**19**)	115—116	−29.1	0.00005	8	Isobauerenol (**40**)			0.0232	6
Lupenone (**20**)	119—120		0.0004	5	Butyrospermol (**41**)			0.00003	7
Germanicone (**21**)	183—184		0.0002	5	Tirucalla-7,21-dien-3β-ol (**42**)			0.0002	8

a) Yield from the dried materials after removal of water by azeotropic distillation.

plates. The HR-MS of **5** and **7** indicated M^+ at *m/z* 468.4004 (Calcd 468.3967) and *m/z* 468.3935, respectively, suggesting the molecular formula to be $C_{32}H_{52}O_2$. The IR absorptions of **5** and **7** indicated the presence of an acetoxyl group in each. The LR-MS of **5** (relative intensity in parentheses) and **7** (relative intensity in square brackets) showed the same major fragment ions at *m/z* 315 (f), 255 (f′), 301 (g), 241 (g′), 289 (h) and 229 (h′) (Chart 2). These fragment ions are observed characteristically in Δ^7-, Δ^8- and $\Delta^{9(11)}$-3β-yl acetates of fernane and multiflorane skeletons.[3] Of the eight tertiary methyl proton signals observed in the ^{1}H-NMR spectra of **5** and **7** (Table 2), three (H-23, 24 and 25) were very similar to those of fern-9(11)-en-3β-yl acetate (**44**),[2] and fern-8-en-3β-yl acetate (**45**),[2] respectively. The other five methyl signals (H-26–H-30) did not coincide with those of multiflor-9(11)-ene and multiflor-8-ene.[4] The olefinic proton of **5** showed almost the same splitting pattern and chemical shift as that of **44**, whereas **7** showed no olefinic proton signal. The identity of ^{13}C-chemical shifts (Table 3) of the A and B ring moiety in **5** with those of **44**[2] also indicates that the left counterpart of **5** is the same as that of **44**. The relative configuration of **5** was established by the NOESY spectrum. That is, NOE interactions were observed between methyl and methyl or methine groups on the α-side of the molecule (H-23–H-5 and H-8–H-27–H-28–H-29), and on the β-side (H-24–H-25 and H-26–H-18–H-30). On the basis of this and biogenetic considerations (this plant contains **1**), **5** and **7** are presumed to be the migrated gammacerane triterpenoids with a $\Delta^{9(11)}$ and a Δ^8 double bond in the molecule, respectively. The structures of **5** and **7** were confirmed by the following acid-induced rearrangement. Compound **5** was treated with 1 N H_2SO_4–AcOH–C_6H_6 at 20 °C for 15 h to give **7** in a good yield and **7** was also obtained from **1** under the same condition. Thus, **5** and **7** were found to be $\Delta^{9(11)}$ and Δ^8 triterpenoids of a migrated gammacerane skeleton, for which we propose the name pichierane.

Compound **6**, pichierenone, was obtained as colorless plates, and the HR-MS of **6** showed M^+ at *m/z* 424.3728 suggesting the molecular formula $C_{30}H_{48}O$. The IR spectrum of **6** indicated the presence of a carbonyl group, and **6** was identical with the ketone, pichier-9(11)-en-3-one, derived from **5** by hydrolysis followed by CrO_3–pyridine complex oxidation.

Compound **8**, isopichierenol, was obtained as colorless plates, and the HR-MS of **8** indicated M^+ at *m/z* 426.3851, suggesting the molecular formula to be $C_{30}H_{50}O$. The IR spectrum of **8** indicated the presence of a hydroxyl group, and **8** proved to be identical with the alcohol, pichier-8-en-3β-ol, obtained by hydrolysis of **7**.

This is the first report of the triterpenoid having a migrated gammacerane skeleton, pichierane. The 42 triterpenoids obtained from the roots, and fifteen from the aerial parts of *P. hieracioides* subsp. *japonica* are mainly pentacyclic triterpenoids belonging to the lupane, gammacerane, migrated gammacerane, oleanane, migrated oleanane, ursane, and migrated ursane groups, with some tetracyclic compounds.

Experimental

Melting points were measured on a Yanagimoto micro apparatus without correction. Specific rotations were observed in $CHCl_3$ solution (c=0.1—0.8) at 22—24 °C. The ^{1}H- and ^{13}C-NMR spectra were taken at 500 and 270/125 MHz, respectively, by the Fourier-transform (FT) method in $CDCl_3$ solution with tetramethylsilane as an internal standard. MS was recorded (direct inlet) at 30 eV and the relative intensities of peaks were reported with reference to the most intense peak higher than *m/z* 100. HPLC was performed on a C-18 reverse-phase column (8 i.d. × 250 mm, refraction index detector), with CH_3CN or CH_3CN–$CHCl_3$ (9 : 1) as the eluent. Silica gel 60, 230—400 mesh (Merck), and 20% $AgNO_3$-impregnated silica gel were used for column chromatography (CC), with hexane–ether, and hexane–benzene (8 : 2) or hexane–benzene (6 : 4) as eluents.

Plant Material The roots and aerial parts of *P. hieracioides* subsp. *japonica* were collected in June, 1981, at Inagi city, Tokyo, Japan. Voucher specimens have been deposited in the Herbarium of Shôwa College of Pharmaceutical Sciences, Tokyo.

Extraction of the Roots and Separation The fresh roots (8.8 kg) were extracted three times with hexane. The extract was evaporated and the residue (51.4 g) was chromatographed on silica gel with hexane (fr. A),

Table 2. ^{1}H-NMR Spectral Data (500 MHz, $CDCl_3$, δ)

	1	2	3	4	5	6
H-23	0.860	0.981	0.832	1.084	0.842	1.037
H-24	0.841	0.766	0.948	1.029	0.940	1.122
H-25	0.864	0.839	0.853	0.937	1.086	1.308
H-26	0.947	0.947	0.948	0.989	0.702	0.733
H-27	0.974	0.981	0.995	0.989	0.787	0.771
H-28	1.076	1.073	1.075	1.084	0.970	0.965
H-29	1.124	1.122	1.123	1.126	0.768	0.771
H-30	1.055	1.056	1.055	1.060	1.032	1.037
H-3α or β	4.488 (dd, 5.9, 10.6)	3.209 (dd, 4.9, 11.6)	3.390 (dd, 2.8, 2.8)	—	4.479 (dd, 6.7, 9.1)	—
H-5α	0.797 (dd, 1.8, 11.9)	0.690 (dd, 1.9, 11.9)	1.20 (m)	1.32 (m)	1.37 (m)	1.70 (m)
H-8α	—	—	—	—	1.988 (br d, 14.0)	2.021 (br d, 14.7)
H-9α	1.21 (m)	1.19 (m)	1.32 (m)	1.27 (m)	—	—
H-13β	1.51 (m)	1.50 (m)	1.48 (m)	1.52 (m)	—	—
H-18β	—	—	—	—	1.724 (dd, 2.8, 12.2)	1.73 (m)
C=C$\underline{H}$–	5.441 (dd, 3.0, 4.9)	5.443 (dd, 3.1, 4.9)	5.443 (dd, 3.1, 4.9)	5.449 (dd, 3.2, 4.7)	5.305 (ddd, 2.6, 2.6, 5.4)	5.375 (ddd, 2.5, 2.5, 5.2)
$C\underline{H}_3COO$–	2.047	—	—	—	2.048	—

	7	8	9	29	30	43
H-23	0.878	0.998	0.846	0.960	0.965	0.847
H-24	0.870	0.798	0.927	0.868	0.855	0.833
H-25	0.972	0.950	0.764	1.067	0.743	0.842
H-26	0.956	0.959	0.976	0.704	0.977	0.962
H-27	0.745	0.757	0.888	0.796	0.890	0.948
H-28	0.983	0.983	0.941	0.970	0.941	0.813
H-29	0.776	0.776	0.777	0.769	0.777	0.791
H-30	1.042	1.044	1.038	1.034	1.039	0.840
H-3α or β	4.492 (dd, 4.5, 11.9)	3.233 (dd, 4.5, 11.8)	4.510 (dd, 4.3, 11.0)	3.206 (dd, 5.8, 10.0)	3.231 (dd, 4.1, 11.5)	4.476 (dd, 6.0, 10.5)
H-5α	1.16 (m)	1.06 (m)	1.41 (m)	1.27 (m)	1.31 (m)	
H-8α	—	—	—	1.985 (br d, 14.1)	—	—
H-9α	—	—	2.30 (m)	—	2.29 (m)	
H-13β	—	—	—	—	—	
H-18β	1.72 (m)	1.72 (m)	1.68 (m)	1.730 (dd, 2.8, 12.3)	1.683 (dd, 2.9, 12.4)	—
C=C$\underline{H}$–	—	—	5.375 (ddd, 3.1, 3.1, 3.6)	5.305 (ddd, 2.5, 2.5, 5.2)	5.382 (ddd, 3.3, 3.3, 3.7)	—
$C\underline{H}_3COO$–	2.051	—	2.054	—	—	2.042

Signals, unless otherwise stated, are 3H, singlet. Multiplicity and coupling constants (J) are shown in parentheses.

hexane–benzene (8 : 2) (frs. B—E), hexane–benzene (1 : 1) (frs. F—H), benzene (fr. I), benzene–ether (9 : 1) (frs. J, K), benzene–ether (1 : 1) (frs. L, M), and ether (fr. N) to give fourteen fractions.

Gammacer-16-en-3β-yl Acetate (1), Pichierenyl Acetate (5), Isopichierenyl Acetate (7), Swertenyl Acetate (9), Lupenyl Acetate (10), Germanicyl Acetate (11), β-Amyrin Acetate (12), Taraxasteryl Acetate (13), ψ-Taraxasteryl Acetate (14), α-Amyrin Acetate (15), Bauerenyl Acetate (16), Isobauerenyl Acetate (17), Butyrospermyl Acetate (18), and Tirucalla-7,21-dien-3β-yl Acetate (19) Fraction E was repeatedly chromatographed on 20% $AgNO_3$-impregnated silica gel with hexane–benzene (8 : 2) followed by HPLC with CH_3CN–$CHCl_3$ (9 : 1) to give the following crystalline solids (recrystallized from acetone to obtain pure specimens). **1** (285 mg). IR ν_{max}^{KBr} cm^{-1}: 1730, 1250. **5** (19 mg). IR ν_{max}^{KBr} cm^{-1}: 1734, 1248. **7** (4 mg). IR ν_{max}^{KBr} cm^{-1}: 1730, 1247. **9** (11 mg). IR ν_{max}^{KBr} cm^{-1}: 1733, 1245. **10** (606 mg). ^{1}H-NMR δ: 0.838 (H-23), 0.838 (H-24), 0.856 (H-25), 1.027 (H-26), 0.936 (H-27), 0.784 (H-28), 4.563, 4.682 (H-29), 1.681 (H-30), 4.464 (dd, J=6.1, 8.3 Hz, H-3), 2.036 (–$OCOC\underline{H}_3$). **11** (349 mg). ^{1}H-NMR δ: 0.843 (H-23), 0.843 (H-24), 0.902 (H-25), 1.073 (H-26), 0.730 (H-27), 1.014 (H-28), 0.938 (H-29), 0.938 (H-30), 4.483 (dd, J=7.1, 9.1 Hz, H-3), 4.862 (d, J=1.2 Hz, H-19), 2.046 (–$OCOC\underline{H}_3$). **12** (974 mg). ^{1}H-NMR δ: 0.867 (H-23), 0.867 (H-24), 0.963 (H-25), 0.963 (H-26), 1.127 (H-27), 0.826 (H-28), 0.867 (H-29), 0.867 (H-30), 4.500 (dd, J=6.7, 8.9 Hz, H-3), 5.177 (dd, J=3.5, 3.5 Hz, H-12), 2.044 (–$OCOC\underline{H}_3$). **13** (2.65 g). ^{1}H-NMR δ: 0.845 (H-23), 0.845 (H-24), 0.872 (H-25), 1.017 (H-26), 0.924 (H-27), 0.845 (H-28), 1.016 (d, J=6.6 Hz, H-29), 4.605 (d, J=2.0 Hz, H-30), 4.484 (dd, J=6.6, 9.6 Hz, H-3), 2.044 (–$OCOC\underline{H}_3$). **14** (624 mg). ^{1}H-NMR δ: 0.848 (H-23), 0.848 (H-24), 0.879 (H-25), 1.046 (H-26), 0.951 (H-27), 0.735 (H-28), 0.987 (d, J=7.0 Hz, H-29), 1.642 (H-30), 4.486 (dd, J=6.7, 9.2 Hz, H-3), 5.263 (bd, J=6.4 Hz, H-21), 2.039 (–$OCOC\underline{H}_3$). **15** (1.82 g). ^{1}H-NMR δ: 0.870 (H-23), 0.870 (H-24), 0.978 (H-25), 1.004 (H-26), 1.063 (H-27), 0.796 (H-28), 0.796 (d, J=5.8 Hz, H-29), 0.913 (br s, H-30), 4.504 (dd, J=6.7, 8.9 Hz, H-3), 5.123 (dd, J=3.5, 3.5 Hz, H-12), 2.046 (–$OCOC\underline{H}_3$). **16** (433 mg). ^{1}H-NMR δ: 0.848 (H-23), 0.931 (H-24), 0.770 (H-25), 0.995 (H-26), 0.946 (H-27), 1.037 (H-28), 1.032 (d, J=7.2 Hz, H-29), 0.912 (d, J=6.9 Hz, H-30), 4.518 (dd, J=5.4, 9.8 Hz, H-3), 5.413 (ddd, J=3.2, 3.2, 3.7 Hz, H-7), 2.050 (–$OCOC\underline{H}_3$). **17** (231 mg). ^{1}H-NMR δ: 0.879 (H-23), 0.872 (H-24), 0.976 (H-25), 1.001 (H-26), 0.838 (H-27), 1.049 (H-28), 0.987 (d, J=5.8 Hz, H-29), 0.900 (d, J=6.1 Hz, H-30), 4.496 (dd, J=5.0, 11.4 Hz, H-3), 2.054 (–$OCOC\underline{H}_3$). **18** (4 mg). ^{1}H-NMR δ: 0.851 (H-23), 0.933 (H-24), 0.764 (H-25), 0.973 (H-26), 0.803 (H-27), 0.846 (d, J=6.1 Hz, H-28), 1.605 (H-29), 1.686 (H-30), 4.516 (dd, J=7.0, 8.7 Hz, H-3), 5.252

Table 3. ^{13}C-NMR Spectral Data (125 MHz, $CDCl_3$, δ)

	1	2	3	4	5	6	7	8	9	29	30
C-1	38.36	38.69	33.26	39.51	38.97	40.50	35.07	35.38	36.46	39.35	36.84
C-2	23.70	27.39	25.39	34.17	24.63	35.15	24.28	27.98	24.20	28.15	27.71
C-3	81.02	79.06	76.26	218.31	81.04	217.02	80.97	79.01	81.17	79.16	79.30
C-4	37.79	38.86	37.63	47.31	38.10	48.10	37.76	38.84	37.79	39.24	38.93
C-5	55.30	55.22	49.00	54.83	44.45	46.43	50.45	50.36	50.74	44.30	50.61
C-6	18.07	18.18	18.14	19.58	18.92	19.28	18.98	19.13	23.95	19.13	24.15
C-7	33.40	33.49	33.34	32.88	17.34	17.37	19.53	19.54	115.95	17.46	116.15
C-8	41.26	41.25	41.46	41.18	40.16	40.02	133.95	134.07	145.10	40.22	145.03
C-9	50.30	50.42	50.22	49.77	150.07	148.83	134.39	134.29	47.64	150.36	47.75
C-10	36.93	37.03	37.16	36.73	37.49	37.53	37.33	37.47	37.49	37.64	35.25
C-11	21.34	21.33	21.21	21.89	116.64	117.36	26.93	27.07	16.65	116.42	16.67
C-12	22.58	22.62	22.65	22.70	37.44	37.42	30.25	30.25	33.87	37.49	33.95
C-13	46.48	46.50	46.49	46.63	37.15	37.20	37.10	37.11	36.46	37.19	36.46
C-14	39.40	39.40	39.52	39.47	37.05	37.16	40.53	40.54	40.98	37.09	40.98
C-15	33.34	33.36	33.34	33.31	27.95	27.98	25.73	25.71	29.09	27.97	29.09
C-16	117.77	117.82	117.86	117.70	27.79	27.77	27.90	27.91	27.84	27.80	27.84
C-17	147.68	147.65	147.66	147.63	38.10	38.11	38.36	38.36	38.12	38.11	38.13
C-18	37.62	37.63	37.54	37.67	41.08	41.09	41.33	41.30	43.33	41.09	43.35
C-19	41.52	41.52	41.53	41.55	21.68	21.70	21.70	21.69	21.53	21.70	21.56
C-20	18.66	18.66	18.68	18.65	23.26	23.25	23.19	23.19	23.23	23.26	23.25
C-21	41.76	41.75	41.78	41.75	37.15	37.12	36.89	36.89	37.08	37.16	37.10
C-22	36.13	36.14	36.14	36.16	39.20	39.20	39.10	39.10	38.96	39.22	38.97
C-23	28.03	28.07	22.22	26.80	27.37	24.30	27.98	28.04	27.49	27.45	27.53
C-24	16.59	15.46	28.29	21.11	16.14	21.67	16.61	15.53	16.02	15.05	14.63
C-25	16.23	16.17	15.98	16.11	25.24	24.19	20.15	20.09	12.93	25.21	12.90
C-26	16.88	16.89	16.90	16.76	15.20	15.23	22.21	22.23	23.74	15.21	23.73
C-27	17.50	17.54	17.69	17.42	16.45	16.50	16.22	16.29	22.14	16.54	22.15
C-28	20.63	20.62	20.65	20.60	16.29	16.30	16.95	16.97	15.76	16.32	16.04
C-29	29.86	29.86	29.88	29.83	25.24	25.24	25.29	25.29	25.33	25.25	25.34
C-30	33.49	33.49	33.50	33.48	22.95	22.94	22.83	22.83	22.98	22.95	22.98

Acetyl signals were observed at δ 21.34, 171.05 in **1**, δ 21.34, 171.04 in **5**, δ 21.35, 171.07 in **7**, δ 21.34, 171.03 in **9**.

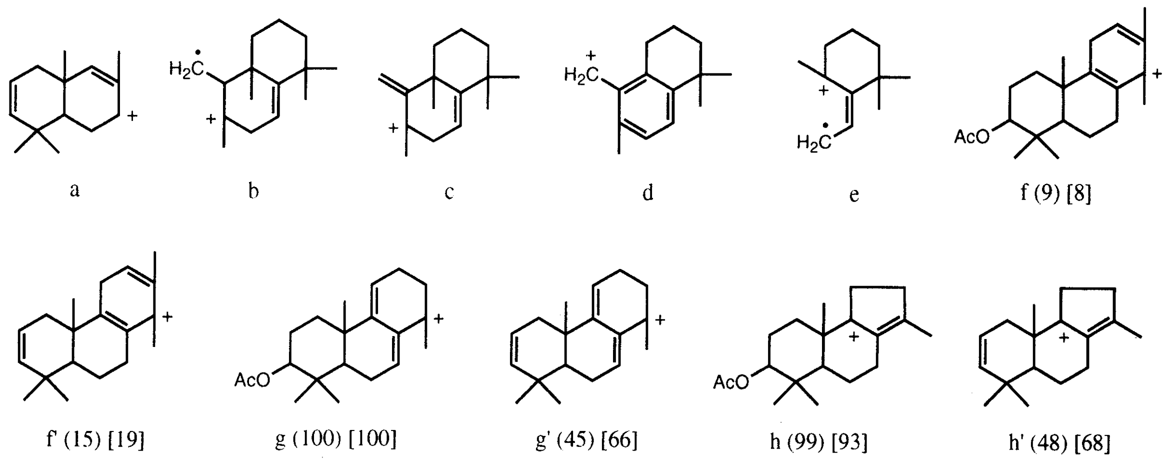


Chart 2

(ddd, J=3.1, 3.1, 3.7 Hz, H-7), 5.093 (br t, J=7.0 Hz, H-21), 2.056 (–OCOC$\underline{H}_3$). **19** (1 mg). ^{1}H-NMR δ: 0.850 (H-23), 0.933 (H-24), 0.767 (H-25), 0.965 (H-26), 0.803 (H-27), 0.885 (d, J=7.0 Hz, H-28), 1.607 (H-29), 1.679 (H-30), 4.515 (dd, J=6.1, 9.1 Hz, H-3), 5.246 (ddd, J=3.1, 3.1, 3.7 Hz, H-7), 5.098 (br t, J=7.0 Hz, H-21), 2.056 (–OCOC$\underline{H}_3$). Compounds **10**—**19** were identified by comparison of their melting point and ^{1}H-NMR data with published values.[4–8)]

Gammacer-16-en-3-one (4), Pichierenone (6), Lupenone (20), Germanicone (21), β-Amyrenone (22), Friedelin (23), Taraxasterone (24), ψ-Taraxasterone (25), α-Amyrenone (26), Bauerenone (27), and Isobauerenone (28) Fraction F was chromatographed repeatedly on 20% $AgNO_3$-impregnated silica gel with hexane–benzene (6 : 4) followed by HPLC with CH_3CN to give the following crystalline solids (recrystallized from acetone to obtain pure specimens). **4** (8 mg). IR ν_{max}^{KBr} cm^{-1}: 1708. **6** (3 mg). IR ν_{max}^{KBr} cm^{-1}: 1709. **20** (9 mg). ^{1}H-NMR δ: 1.068 (H-23), 1.024 (H-24), 0.928 (H-25), 1.068 (H-26), 0.953 (H-27), 0.796 (H-28), 4.566, 4.689 (H-29), 1.679 (H-30). **21** (5 mg). ^{1}H-NMR δ: 1.078 (H-23), 1.029 (H-24), 0.960 (H-25), 1.105 (H-26), 0.747 (H-27), 1.029 (H-28), 0.941 (H-29), 0.941 (H-30), 4.864 (d, J=1.2 Hz, H-19). **22** (14 mg). ^{1}H-NMR δ: 1.095 (H-23), 1.056 (H-24), 1.056 (H-25), 1.022 (H-26), 1.142 (H-27), 0.840 (H-28), 0.872 (H-29), 0.872 (H-30), 5.203 (dd, J=3.4, 3.4 Hz, H-12). **23** (1 mg). MS m/z: 426, 411, 341, 273, 259, 247, 218, 205, 191. **24** (35 mg). ^{1}H-NMR δ: 1.076 (H-23), 1.031 (H-24), 0.946 (H-25), 1.056 (H-26), 0.946 (H-27), 0.865 (H-28), 1.022 (d, J=6.8 Hz, H-29), 4.605, 4.625 (H-30). **25** (14 mg), ^{1}H-NMR δ: 1.080 (H-23), 1.031 (H-24), 0.955 (H-25), 1.080 (H-26), 0.955 (H-27), 0.745 (H-28), 0.993 (d, J=7.6 Hz, H-29), 1.644 (H-30), 5.277 (br d, J=6.4 Hz, H-21). **26** (22 mg). ^{1}H-NMR δ: 1.080 (H-23), 1.058 (H-24), 1.080 (H-25), 1.058 (H-26), 1.080 (H-27), 0.811 (H-28), 0.795 (d, J=5.6 Hz, H-29), 0.915 (br s, H-30), 5.152 (dd, J=3.7, 3.7 Hz, H-12). **27** (1 mg). MS m/z: 424, 409, 271, 257, 245. **28** (6 mg). ^{1}H-NMR δ: 1.083 (H-23), 1.056 (H-24), 1.056 (H-25), 1.022 (H-26), 0.848 (H-27), 1.056 (H-28), 0.895 (d,

$J=5.9$ Hz, H-29), 1.044 (d, $J=6.8$ Hz, H-30). Compounds **20**—**28** were identified by comparison of their melting point and ^{1}H-NMR and MS data with published values.[3,5,6]

Gammacer-16-en-3α-ol (3) Fraction G was chromatographed on silica gel followed by recrystallization from acetone to give **3** (1.43 g). IR ν_{max}^{KBr} cm^{-1}: 3510, 1060.

Gammacer-16-en-3β-ol (2), Pichierenol (29), Isopichierenol (8), Swertenol (30), Neomotiol (31), Lupeol (32), Germanicol (33), β-Amyrin (34), Multiflorenol (35), Taraxasterol (36), ψ-Taraxasterol (37), α-Amyrin (38), Bauerenol (39), Isobauerenol (40), Butyrospermol (41), and Tirucalla-7,21-dien-3β-ol (42) Fraction I was acetylated with acetic anhydride–pyridine. This acetate mixture was purified and identified by the same method as fraction E (acetate fraction). Gammacer-16-en-3β-yl acetate (**2a**, 20 mg), mp 287—288 °C. Pichierenyl acetate (**29a**, 6 mg), mp 271—272 °C. Isopichierenyl acetate (**8a**, 1 mg). Swertenyl acetate (**30a**, 1 mg). Neomotiyl acetate (**31a**, 0.5 mg). MS *m/z*: 468, 453, 408, 393, 218, 203, 189, 175. Lupenyl acetate (**32a**, 496 mg), mp 219—221 °C. Germanicyl acetate (**33a**, 7 mg), mp 279—281 °C. β-Amyrin acetate (**34a**, 184 mg), mp 246—247 °C. Multiflorenyl acetate (**35a**, 28 mg), mp 226—227 °C. ^{1}H-NMR δ: 0.857 (H-23), 0.938 (H-24), 0.762 (H-25), 1.071 (H-26), 1.071 (H-27), 1.056 (H-28), 0.970 (H-29), 0.970 (H-30), 4.503 (dd, $J=5.7$, 9.8 Hz, H-3), 5.465 (ddd, $J=3.0$, 3.0, 3.4 Hz, H-7), 2.054 (–OCO$C\underline{H}_3$). Taraxasteryl acetate (**36a**, 170 mg), mp 247—249 °C. ψ-Taraxasteryl acetate (**37a**, 85 mg), mp 237—238 °C. α-Amyrin acetate (**38a**, 51 mg), mp 226—228 °C. Bauerenyl acetate (**39a**, 261 mg), mp 294—296 °C. Isobauerenyl acetate (**40a**, 478 mg), mp 226—228 °C. Butyrospermyl acetate (**41a**, 1 mg). Tirucalla-7,21-dien-3β-yl acetate (**42a**, 5 mg). Compounds **29a**—**42a** were identified by comparison of their melting point and ^{1}H-NMR and MS data with published values.[3-11]

Extraction of the Fresh Aerial Parts The fresh aerial parts (12.3 kg) were extracted three times with hexane. The extract was evaporated and the residue (68.7 g) was chromatographed on silica gel with hexane–benzene (8 : 2) (frs. A′—G′), hexane–benzene (1 : 1) (fr. H′), benzene (frs. I′—K′), benzene–ether (9 : 1) (frs. L′, M′) and ether (fr. N′) to give fourteen fractions.

Lupenyl Acetate (10), Germanicyl Acetate (11), β-Amyrin Acetate (12), Taraxasteryl Acetate (13), ψ-Taraxasteryl Acetate (14), α-Amyrin Acetate (15), Bauerenyl Acetate (16), and Isobauerenyl Acetate (17) Fraction G′ was chromatographed repeatedly on 20% $AgNO_3$-impregnated silica gel to give the following crystalline solids (recrystallized from acetone to obtain pure specimens). **10** (976 mg), mp 219—221 °C. **11** (393 mg), mp 278—280 °C. **12** (763 mg), mp 244—246 °C. **13** (1.56 g), mp 249—251 °C. **14** (486 mg), mp 235—237 °C. **15** (825 mg), mp 226—228 °C. **16** (53 mg), mp 293—294 °C. **17** (469 mg), mp 226—228 °C. Compounds **10**—**17** were identified by comparison of their melting point and ^{1}H-NMR data with published values.[6,7]

Lupeol (32), Germanicol (33), β-Amyrin (34), Taraxasterol (36), α-Amyrin (38), Bauerenol (39), and Isobauerenol (40) Fraction I′ was acetylated with acetic anhydride–pyridine. This acetate mixture was purified and identified by the same method as used for fraction G′ (acetate fraction). Lupenyl acetate (**32a**, 509 mg), mp 218—221 °C. Germanicyl acetate (**33a**, 60 mg), mp 277—278 °C. β-Amyrin acetate (**34a**, 567 mg), mp 245.5—247 °C. Taraxasteryl acetate (**36a**, 495 mg), mp 248—250 °C. α-Amyrin acetate (**38a**, 402 mg), mp 227—229 °C. Bauerenyl acetate (**39a**, 43 mg), mp 287.5—288.5 °C. Isobauerenyl acetate (**40a**, 382 mg), mp 227—229 °C. Compounds **32a**—**34a**, **36a**, and **38a**—**40a** were identified by comparison of their melting point and ^{1}H-NMR data with published values.[6,7]

Hydrolysis of Gammacer-16-en-3β-yl Acetate (1) **1** (10 mg) was refluxed with 5% KOH–EtOH for 1 h, and the product was chromatographed on silica gel, then recrystallized from MeOH to give **2** (9 mg). IR ν_{max}^{KBr} cm^{-1}: 3380, 1041.

Oxidation of Gammacer-16-en-3β-ol (2) **2** (9 mg) was treated with CrO_3–pyridine complex overnight at room temperature, and the product was chromatographed on silica gel, then recrystallized from acetone to give **4** (5 mg).

Oxidation of Gammacer-16-en-3α-ol (3) **3** (10 mg) was treated with CrO_3–pyridine complex overnight at room temperature, and the product was chromatographed on silica gel, then recrystallized from acetone to give **4** (7 mg).

Acid Induced Rearrangement of Pichierenyl Acetate (5) **5** (3 mg) was treated with 1 N H_2SO_4–AcOH–C_6H_6 at 20 °C for 15 h under N_2 gas. The product was chromatographed on silica gel, and the crystalline product obtained from the hexane–benzene (8 : 2) eluate was found to be identical (^{1}H-NMR) with **7** (1 mg).

Acid Induced Rearrangement of Gammacer-16-en-3β-yl Acetate (1) **1** (18 mg) was treated with 1 N H_2SO_4–AcOH–C_6H_6 at 20 °C for 18 h under N_2 gas. The product (16 mg) was separated by HPLC with CH_3CN–$CHCl_3$ (9 : 1) to give **7** (2 mg).

Hydrolysis of Pichierenyl Acetate (5) **5** (2 mg) was treated with 5% KOH–EtOH in the same manner as mentioned above. The product was chromatographed on silica gel, and the crystalline product from benzene eluate was recrystallized from MeOH to give **29** (1.5 mg). IR ν_{max}^{KBr} cm^{-1}: 3390, 1041, 1024.

Oxidation of Pichierenol (29) **29** (1.5 mg) was treated with CrO_3–pyridine complex in the same manner as mentioned above. The product was chromatographed on silica gel, and the crystalline product was recrystallized from acetone to give **6** (0.8 mg).

Hydrolysis of Isopichierenyl Acetate (7) **7** (3 mg) was treated with 5% KOH–EtOH in the same manner as mentioned above. The product was chromatographed on silica gel, and the crystalline product from the benzene eluate was recrystallized from MeOH to give **8** (1 mg). IR ν_{max}^{KBr} cm^{-1}: 3400, 1030, 1022.

Hydrolysis of Swertenyl Acetate (9) **9** (5 mg) was treated with 5% KOH–EtOH in the same manner as mentioned above. The product was chromatographed on silica gel, and the crystalline product from the benzene eluate was recrystallized from MeOH to give **30** (4 mg). IR ν_{max}^{KBr} cm^{-1}: 3430, 1027.

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