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# Studies on the Constituents of *Baccharis genistelloides*

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A tetra-nor clerodane-type diterpene (**4a**) and an aromatic ester (**10**) were isolated from samples of *Baccharis genistelloides* (Compositae) collected in summer and winter in Brazil, along with three known diterpenes and six flavonoids. The structures were elucidated on the basis of spectral evidence.

**Keywords**——*Baccharis genistelloides*; Compositae; clerodane-type diterpene; tetra-nor diterpene; bitter substance; 3,4-dimethoxycinnamoyl benzoate; flavonoid

*Baccharis genistelloides* (Compositae) is called "Carqueja" and widely used as a folk medicine in Brazil. We obtained samples collected in summer (February) and winter (August) in Brazil, and examined their constituents. The summer material contained a new clerodane-type tetra-nor diterpene (**4a**) along with three known diterpenes, **1**[1], **2**[1] and **3**,[2] and five flavones, eupatrin (**5**),[3] cirsimaritin (**6**),[4] cirsiliol (**7**),[5] apigenin (**8**)[6] and genkwanin (**9**).[7] The winter material gave a new aromatic ester (**10**) along with **8**, **9** and eriodictyol (**11**).[8] This paper deals with the chemical structures of these compounds.

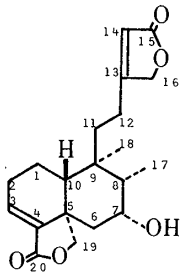
The ethyl acetate(AcOEt)-soluble fraction of the summer material was treated as described in the experimental section to give nine compounds, **1**—**9**. The winter sample was treated in the same way to give four compounds, **8**—**11**.

The structures of **1**, mp 198—202 °C, $C_{20}H_{26}O_5$, **2**, mp 198—206 °C, $C_{20}H_{28}O_5$, and **3**, viscous oil, $C_{20}H_{26}O_5$, were presumed on the basis of physicochemical and spectral data and confirmed by comparing the data with reported data.

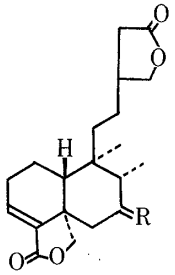
Compound **4a**, mp 253—258 °C, did not give a molecular ion ($M^+$), but gave a fragment, *m/z* 264 ($M^+ - CH_2O$) in the mass spectrum (MS). The molecular formula of **4a** was established to be $C_{16}H_{22}O_5$ by elemental analysis, and the carbon nuclear magnetic resonance ($^{13}$C-NMR) spectrum supported this, showing sixteen peaks. The infrared (IR) and $^{13}$C-NMR spectra showed the presence of a carboxyl group (3380 $cm^{-1}$, $\delta$ 174.3), which was further confirmed by the formation of a methyl ester (**4b**), mp 125—127 °C, MS *m/z*; 308 ($M^+$) ($C_{17}H_{24}O_5$) of **4a**. The proton nuclear magnetic resonance ($^1$H-NMR) spectrum of **4a** showed the presence of secondary methyl ($\delta$ 1.18, $J$ = 6.8 Hz) and tertiary methyl ($\delta$ 0.95) groups and the characteristic $\alpha,\beta$-unsaturated-$\gamma$-lactone moiety, which gave characteristic signal patterns, $\delta$ 6.73 (H, dd, $J$ = 6.6, 2.6 Hz, 3-H), 3.98 (H, dd, $J$ = 7.9, 1.8 Hz, 19-$H_\alpha$) and 4.56 (H, d, $J$ = 7.9 Hz, 19-$H_\beta$). The $H_\alpha$ signal of $CH_2$ at C-19 coupled with $H_\beta$ of $CH_2$ at C-6 through the W-effect and $H_\beta$ was shifted to lower field by the effect of the $\alpha$-OH group at C-7. From these data **4a** was established to be the tetra-nor diterpene having the same A and B-ring part as **1** and **2**. This structure was supported by a comparison of the $^1$H-NMR and $^{13}$C-NMR spectra of **1**, **2**, **3** and **4a** ($^{13}$C-NMR data are shown in Table I).

Compounds **5**, **6**, **7**, **8** and **9** were identified as eupatrin, cirsimaritin, cirsiliol, apigenin and genkwanin, respectively, by comparing their physicochemical and spectral data with the reported data.

Compound **10**, mp 69 °C, $C_{18}H_{18}O_4$, has an ester group (1725 $cm^{-1}$). The $^1$H-NMR and $^{13}$C-NMR spectra of **10** showed the presence of two methoxyl groups ($\delta$ 3.87, 3.89, and $\delta$

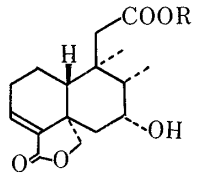


**1**

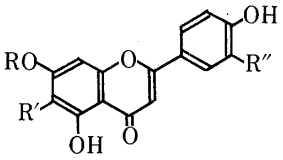


**2** : R=<OH H

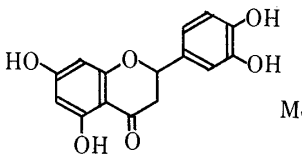
**3** : R=O



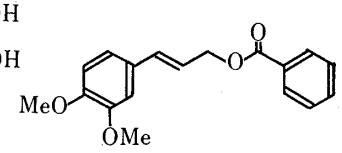
**4a** : R=H
**4b** : R=Me



**5** : R=Me, R′=OMe, R″=OMe
**6** : R=Me, R′=OMe, R″=H
**7** : R=Me, R′=OMe, R″=OH
**8** : R=H, R′=H, R″=H
**9** : R=Me, R′=H, R″=H



**11**



**10**

Chart 1

TABLE I. $^{13}$C-NMR Data for the Diterpenes

| Carbon | **1**[a] | **2**[a] | **3**[a] | **4a**[b] | **4b**[a] |
|---|---|---|---|---|---|
| 1 | 19.4 | 19.3 | 20.8 | 20.5 | 20.2 |
| 2 | 27.7 | 27.6 | 27.3 | 28.0 | 27.7 |
| 3 | 134.8 | 134.9 | 136.9 | 136.7 | 135.4 |
| 4 | 139.3 | 139.2 | 136.2 | 139.4 | 138.6 |
| 5 | 45.0 | 45.0 | 48.0 | 45.7 | 45.0 |
| 6 | 40.7 | 40.5 | 50.5 | 40.7 | 40.3 |
| 7 | 72.2 | 72.1 | 208.9 | 72.2 | 72.6 |
| 8 | 40.6 | 40.5 | 51.4 | 42.1 | 41.8 |
| 9 | 38.6 | 38.4 | 43.6 | 40.4 | 40.3 |
| 10 | 48.5 | 48.2 | 47.8 | 49.2 | 48.9 |
| 11 | 35.7 | 36.2 | 35.7 | 43.2 | 42.8 |
| 12 | 22.3 | 26.4 | 26.8 | 174.3 | 171.7 |
| 13 | 169.6 | 30.7 | 35.9 | — | — |
| 14 | 115.4 | 34.6 | 34.5 | — | — |
| 15 | 173.7 | 176.9 | 176.2 | — | — |
| 16 | 73.0 | 73.3 | 71.1 | — | — |
| 17 | 12.0 | 11.9 | 7.7 | 12.5 | 12.1 |
| 18 | 19.1 | 19.2 | 18.9 | 18.9 | 18.7 |
| 19 | 72.7 | 72.8 | 73.0 | 73.6 | 72.6 |
| 20 | 170.1 | 170.1 | 167.6 | 171.8 | 169.7 |

*a*) Measured in $CDCl_3$. *b*) Measured in $CDCl_3+CD_3OD$.

56.1). Furthermore, the $^1$H-NMR spectrum of **10** showed the presence of a phenyl group [$\delta$ 7.3—7.6 (3H, m) and 8.08 (2H, dd, $J$=7.4, 1.8 Hz)], a 3,4-dimethoxyphenyl group [$\delta$ 6.93 (H, dd, $J$=8.5, 1.7 Hz), 6.80 (H, d, $J$=8.5 Hz), 6.97 (H, d, $J$=1.7 Hz)] and a Ph–CH=CH–$CH_2$–O– group [$\delta$ 6.70 (H, d, $J$=15.9 Hz), 6.26 (H, dt, $J$=15.9, 6.1 Hz), 4.97 (2H, d, $J$=6.1 Hz)]. The MS of **10** gave fragments, $m/z$ 105 ($C_7H_5O$) (base peak) and 193 ($C_{11}H_{13}O_3$), which were in accord with the structure, having methoxyl groups substituted on the alcohol part. The $^{13}$C-NMR spectrum also supported the structure of **10**, which was confirmed by synthesis of **10** from ethyl 3,4-dimethoxycinnamate as described in the experimental section.

Compound **11** was identified as eriodictyol by comparing the spectral data, with the reported values.

The summer material had a bitter taste, whereas the winter one had no taste at all. Some of the clerodane-type diterpenes isolated from the summer sample showed a bitter taste. The seasonal difference of the constituents is interesting.

## Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO A-202 grating infrared spectrometer. UV spectra were recorded on a Hitachi model 200-10 spectrometer. Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a JEOL JNM-FX 90Q FT spectrometer with tetramethylsilane as an internal standard ($\delta$ value). MS were recorded on JEOL JMS D-100 and JEOL JMS 01SG-2 mass spectrometers. Thin layer chromatography (TLC) was performed on precoated Silica gel $60F_{254}$ plates (Merck). Preparative layer chromatography (PLC) was performed on Silica gel $PF_{254}$ plates (Merck, 200×200×0.7 mm). Column chromatography was performed on Silica gel type 60 (Merck).

**Isolation of the Constituents**——The dried aerial part of *B. genistelloides* (2.5 kg), collected in summer (February) in Brazil, was extracted with hot MeOH. The MeOH extract was fractionated between AcOEt and water to give an AcOEt-soluble fraction. The AcOEt fraction (200 g) was chromatographed on an $SiO_2$ column with a $CHCl_3$–MeOH gradient to give many fractions. The fractions were recombined into six fractions, frs. I—VI. Fraction III gave eupatrin (**5**) (16 g) on filtration. The mother liquor was repeatedly chromatographed to give **5** (450 mg), **1** (2.5 g), **2**(5.6 g), cirsimaritin (**6**) (130 mg), and **3** (140 mg). Fraction IV gave **1** (190 mg), **2** (500 mg), **6** (150 mg) and genkwanin (**9**) (120 mg). Fraction V gave **4a** (90 mg), **6** (250 mg), cirsiliol (**7**) (130 mg) and **9** (50 mg). Fraction VI gave **7** (80 mg) and apigenin (**8**) (200 mg).

The dried winter material (2 kg) was extracted with hot MeOH to give the extract, which was treated in the same way as the summer material. The AcOEt fraction was chromatographed on an $SiO_2$ column and subjected to pTLC to give compound **10** (70 mg), eriodictyol (**11**) (100 mg), **8** (1 g) and **9** (870 mg).

**Compound 1**——Colorless prisms, mp 198—202 °C (AcOEt). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3490, 1735, 1654, 1635, 1230, 1208, 1170. UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 204 (4.02). 242 (2.99), $[\alpha]_D^{20}$ −150.9° ($c$=2.0, MeOH). $^1$H-NMR ($CDCl_3$): 0.92 (3H, s, 18-$CH_3$), 1.05 (3H, d, $J$=7.0 Hz, 17-$CH_3$), 1.2—1.9 (6H, m, 1-H, 6-H, 11-H), 1.9—2.6 (6H, m, 2-H, 8-H, 10-H, 12-H), 3.92 (H, dd, $J$=7.9, 1.8 Hz, 19-H), 4.13 (H, m, 7-H), 4.78 (2H, d, $J$=1.8 Hz, 16-H), 5.35 (H, d, $J$=7.6 Hz, 19-H), 5.87 (H, t, $J$=1.8 Hz, 14-H), 6.70 (H, dd, $J$=6.8, 2.8 Hz, 3-H). $^{13}$C-NMR as given in Table I. MS $m/z$: 346.1776 ($M^+$) (Calcd for $C_{20}H_{26}O_5$, 346.1783).

**Compound 2**——Colorless prisms, 199—206 °C (AcOEt–$CHCl_3$). IR $\nu_{max}^{KBr}$ cm$^{-1}$:3510, 1768, 1740, 1660, 1198, 1180, 1063. UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 205 (3.96), 245 (2.88), $[\alpha]_D^{20}$ −152.0° ($c$=1.5, $CHCl_3$). $^1$H-NMR ($CDCl_3$): 0.86 (3H, s, 18-$CH_3$), 1.02 (3H, d, $J$=6.8 Hz, 17-$CH_3$), 1.2—2.9 (12—13H, m, 1-H, 2-H, 6-H, 8-H, 10-H, 11-H, 12-H, 13-H), 3.90 (H, dd, $J$=7.9, 1.8 Hz, 19-H), 4.1 (H, m, 7-H), 4.47 (H, dd, $J$=9.2, 7.2 Hz, 16-H), 5.34 (H, d, $J$=7.4 Hz, 19-H), 6.69 (H, dd, $J$=6.6, 2.2 Hz, 3-H). $^{13}$C-NMR as given in Table I. *Anal.* Calcd for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found: C, 68.96; H, 8.10.

**Compound 3**——Colorless viscous oil. IR $\nu_{max}^{film}$ cm$^{-1}$: 1775, 1710, 1172, $[\alpha]_D^{20}$ −128.2° ($c$=1.2, $CHCl_3$). $^1$H-NMR ($CDCl_3$): 0.63 (3H, s, 18-$CH_3$), 0.98 (3H, d, $J$=6.8 Hz, 17-$CH_3$), 3.96 (2H, s, 19-H), 6.85 (H, dd, $J$=6.8, 2.8 Hz, 3H), the other signals overlapped in the ranges of 1.1—2.9 and 3.9—4.6. $^{13}$C-NMR as given in Table I. MS $m/z$: 346 ($M^+$) ($C_{20}H_{26}O_5$).

**Compound 4a**——Colorless needles, mp 253—258 °C (AcOEt). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3500, 3380, 1770, 1730, 1380, 1210, 1190, $[\alpha]_D^{20}$ −122.4° ($c$=1.2, MeOH). $^1$H-NMR ($CDCl_3$+$CD_3OD$): 0.95 (3H, s, 18-$CH_3$), 1.18 (3H, d, $J$=6.8 Hz, 17-$CH_3$), 1.2—1.6 (2H, m, 1-H), 1.9—2.4 (6H, m, 2-H, 6-H, 8-H, 10-H), 2.45 (2H, s, 11-H), 3.98 (H, dd, $J$=7.9, 1.8 Hz, 19-$H_\alpha$), 4.01 (H, m, 7-H), 4.56 (H, d, $J$=7.9 Hz, 19-$H_\beta$), 6.73 (H, dd, $J$=6.6, 2.6 Hz, 3-H). $^{13}$C-NMR

as given in Table I. MS *m/z*: 264 ($M^+ - CH_2O$) ($C_{15}H_{20}O_4$). *Anal.* Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.35; H, 7.51.

**Eupatrin (5)**——Pale yellow needles, mp 191—200 °C ($CHCl_3$–MeOH). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2950, 1660, 1610, 1370, 1280, 1200, 1126. MS *m/z*: 344 ($M^+$) (base peak, $C_{18}H_{16}O_7$), identical with the reported data.[3]

**Cirsimaritin (6)**——Light yellow powder, mp 271—272 °C (AcOEt). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3290, 1657, 1600, 1500, 1445, 1362, 1249. MS *m/z*: 314 ($M^+$) (base peak, $C_{17}H_{14}O_6$), identical with the reported data.[4]

**Cirsiliol (7)**——Yellow powder, mp 291—298 °C (MeOH). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420, 1650, 1596, 1480, 1462, 1370, 1281, 1260, 1125. MS *m/z*: 330 ($M^+$) ($C_{17}H_{14}O_7$), identical with the reported data.[5]

**Apigenin (8)**——Pale yellow powder, mp 300 °C (AcOEt–MeOH). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3300—2630, 1660, 1495, 1355, 1265, 1180, 1030. MS *m/z*: 270 ($M^+$) ($C_{15}H_{10}O_5$), identical with the reported data.[6]

**Genkwanin (9)**——Pale yellow powder, mp 290—295 °C (AcOEt–MeOH). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3270, 1665, 1605, 1590, 1380, 1225, 1180, 1164, identical with authentic sample by TLC and IR spectral comparisons.

**Compound (10)**——Colorless needles, mp 69 °C (*n*-hexane). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2975, 1725, 1605, 1515, 1455, 1260, 1140, 1100, 1070. $^1$H-NMR ($CDCl_3$): 3.87, 3.89 (each 3H, s, OMe), 4.97 (2H, d, $J$=6.1 Hz, 3′-H), 6.26 (H, dt, $J$=15.9, 6.1 Hz, 2′-H), 6.70 (H, d, $J$=15.9 Hz, 1′-H), 6.80 (H, d, $J$=8.5 Hz, 5-H), 6.93 (H, dd, $J$=8.5, 1.7 Hz, 6-H), 6.97 (H, d, $J$=1.7 Hz, 2-H), 7.3—7.6 (3H, m, 3″, 4″, 5″-H), 8.08 (2H, dd, $J$=7.4, 1.8 Hz, 2″, 6″-H). $^{13}$C-NMR ($CDCl_3$): 56.1 (q), 65.7 (t), 109.7 (d), 111.7 (d), 120.2 (s), 120.2 (d), 121.5 (d), 128.4 (d), 129.7 (d), 130.5 (s), 132.9 (d), 134.4 (d), 149.4 (s), 149.6 (s), 166.4 (s). MS *m/z*: 298.1207 ($M^+$) (Calcd for $C_{18}H_{18}O_4$, 298.1231).

**Eriodictyol (11)**——Colorless powder, mp 225—228 °C (MeOH). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3550, 1640, 1605, 1450, 1350. MS *m/z*: 288 ($M^+$) ($C_{15}H_{12}O_6$), identical with the reported data.[8]

**Methyl Ester (4b)——4a** was methylated with $CH_2N_2$ to give a methyl ester (**4b**), mp 125—127 °C (*n*-hexane–$CHCl_3$). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450, 1746, 1215, 1190, 1040. $^1$H-NMR ($CDCl_3$): 0.89 (3H, s, 18-$CH_3$), 1.14 (3H, d, $J$=7.0 Hz, 17-$CH_3$), 1.2—2.4 (8H, m, 1-H, 2-H, 6-H, 8-H, 10-H), 2.42 (2H, s, 11-H), 3.66 (3H, s, OMe), 3.90 (H, dd, $J$=7.6, 2.0 Hz, 19-H), 4.07 (H, m, 7-H), 5.27 (H, d, $J$=7.6 Hz, 19-H), 6.73 (H, dd, $J$=6.5, 2.8 Hz, 3-H). $^{13}$C-NMR as given in Table I. MS *m/z*: 308 ($M^+$) ($C_{17}H_{24}O_5$).

**Synthesis of 10 from Ethyl 3,4-Dimethoxycinnamate**——Ethyl 3,4-dimethoxycinnamate was reduced with excess $LiAlH_4$ in dry ether. The reaction solution was worked up as usual to give an alcohol, which was benzoylated to give **10**. This product was recrystallized from *n*-hexane to give colorless needles, mp 69 °C, *Anal.* Calcd for $C_{18}H_{18}O_4$: C, 72.46; H, 6.08. Found: C, 72.76; H, 6.06. The synthetic **10** was identical with the natural product on the basis of mp, IR, TLC and $^1$H-NMR spectral comparisons.

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