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A new flavone glycoside and other constituents from *Carduus crispus*

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Received April 22, 2004, accepted May 20, 2004

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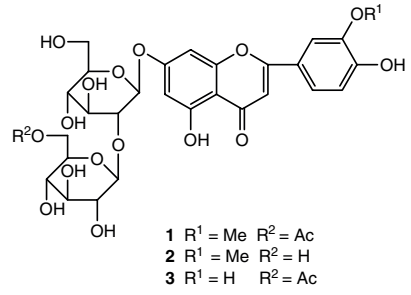
Pharmazie 60: 233–236 (2005)

A new flavone glycoside, chrysoeriol 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-glucopyranosyl-β-D-glucopyranoside (**1**), along with fourteen known compounds **2**–**15** were isolated from the whole plant of *Carduus crispus* Linn. Their structures were established on the basis of spectroscopic methods and chemical evidences. The antitumour activity of compound **1**, **4** and **5** was tested. Compound **4** exhibited significant antitumour activity against HO-8901 (human ovarian neoplasm) cells.

1. Introduction

The genus *Carduus* (Compositae) comprises 95 species all over the world. Three of them are distributed in China (Deletis Flora Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita 1979). Investigations of chemical constitutes showed that flavonoids, alkaloids and coumarins were the main components of this genus. *Carduus crispus* L. is widely distributed in Northwestern China. The whole plants or roots of it were used as folk medicine in China for treatment of rheumatism, urethritis, furuncle and scald (Jiangsu New Medical College, 1977). Isoquinoline alkaloids from this plant collected in Inner Mongolia have been reported (Zhang et al. 2002). In order to find bioactive compounds, we systematically studied the chemical constitutes of this plant which was collected in Zhang county, Gansu province. In this paper, we report the isolation and structural elucidation of a new flavone glycoside, chrysoeriol 7-*O*-(2″-*O*-6″′-*O*-acetyl-β-D-glucopyranosyl-β-D-glucopyranoside (**1**), as well as fourteen known compounds (**2**–**15**). Compounds **2** and **3** were first isolated from this genus. The ^{1}H and ^{13}C NMR spectral data of **2** were first reported. In addition, the antitumour activity of compounds **1**, **4** and **5** are described.



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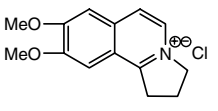
3 R^1 = H R^2 = Ac



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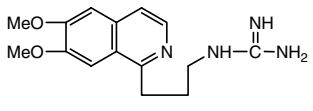

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2. Investigations, results and discussion

From the 95% ethanol extract of the whole plant of *Carduus crispus*, a new flavone glycoside (**1**), was isolated, together with fourteen known compounds: chrysoeriol 7-sophoroside (**2**) (Umber, 1980), linariifolioside (**3**) (Ma et al. 1991), crispine B (**4**) (Zhang et al. 2002), crispine C (**5**) (Zhang et al. 2002), afzelin (**6**) (Matthes et al. 1980), astragaline (**7**) (Markham et al. 1978), luteolin 3′-*O*-α-L-rhamnopyranoside (**8**) (Markham et al. 1985). luteolin (**9**) (Ma, et al. 1991; Wagner et al. 1976), apigenin (**10**) (Shen et al. 1993), tricin (**11**) (Lee et al. 1981), adenosine (**12**) (Chenon et al. 1975), adenine (**13**) (Thorpe et al.1974), syringin (**14**) (Niwa et al. 1988), and (*E*)-2-butenedioc acid (**15**) (Sadtler Research Laboratories Inc 1969). The known compounds were identified by comparison of their spectral data (FABMS, EIMS, ^{1}H and ^{13}C NMR) with those published in the literature.

Compound **1** was isolated as yellow amorphous powder. The UV spectrum showed the typical absorption of flavone at λ_{max} (log ε) = 214 (1.39), 268 (1.12) and 346 (1.51) nm. Its IR spectrum showed hydroxyl absorption at 3700–3300 cm^{-1}, an α,β-unsaturated keton at 1749 and 1657 cm^{-1}, aromatic ring absorption at 1603, 1563 and 1492 cm^{-1}. It was established to have a molecular formula $C_{30}H_{34}O_{17}$, which was deduced by HRESIMS (m/z 667.1870 $[M + H]^+$, calcd. 667.1869). The ^{1}H, ^{13}C NMR had signals for a flavonoid aglycone, an acetyl and two anomeric protons of glycose. Acid hydrolysis of **1** showed the presence of glucose and an aglycone which was identified as chrysoeriol by ^{1}H NMR data (Chen et al. 1989) and TLC comparison with an authentic sample. The position of methoxy further confirmed by NOEDS spectroscopy which revealed that the protons of methoxy had an effect on the H-2′ (3.6%) and no effect on H-5′, suggest-

Table 1: The IC_{50} of compound 1, 4 and 5 on survival rate of the two human tumor cell lines

Compd.	IC_{50} (mg/L^{-1})	
	HO-8910	Bel-7402
1	> 50	> 50
4	20.17 ± 4.50	135.83 ± 7.62
5	> 50	> 50
Vincristine sulfate	20.7 ± 1.9	25.9 ± 3.4

ing the methoxy was attached to C-3′. The two anomeric protons arising from the sugar moieties were at δ_H 5.22 (J = 7.6 Hz) and 4.51 ppm (J =8.4 Hz,), which correlated respectively with signals at δ_C 98.9 and 105.3 ppm in the HMQC. The values of the coupling constant indicated that the two glucose had β configuration. A 1D-TOCSY experiment allowed to identify the spin systems of each sugar residue. In the 1D-TOCSY of the terminal sugar, lower field shifts of H-6‴ (from δ_H 3.96, 3.74 to 4.12, 3.98 ppm) together with the lower field shift of C-6‴ (from δ_C 61.5 to 64.2 ppm) signal suggested that the acetyl was attached to the 6‴-hydroxy. The terminal sugar was identified as 6‴-acetyl-β-D-glucose on the basis of the large coupling contants observed for all the oxymethine protons. In the ^{13}C NMR spectrum, C-7 has 0.8 ppm upfield shift and C-6, C-8, C-10 have 1.4, 1.6, 2.7 ppm downfield shift respectively in comparison with chrysoeriol (Wagner et al, 1976), indicating the inner glucose was attached to the 7-hydroxyl (Markham et al. 1978), which can be further confirmed by the cross peak at δ_H 5.22/δ_C 163.4 (H-1″/C-7) in the HMBC spectrum. The interglucosidic linkage can be deduced from the effect of glycosylation. The dowdfield shift of C-2″ and upfield shift of C-1″ suggested that the two monosaccharides have a 1-2 linkage (Markham et al. 1978). On the basis of these conclusions, the structure of compound **1** was established as chrysoeriol 7-*O*-(2″-*O*-6‴-*O*-acetyl-β-D-glucopyranosyl-β-D-glucopyranoside).

Compounds **1**, **4** and **5** were tested for antitumour activity for human ovarian neoplasm cells HO-8910 and human hepatoma cells Bel-7402. Compound **4** could inhibit the growth of two cell lines, and the effect to HO-8910 cells was more effective. The IC_{50} to HO-8910 cells was 20.17 ± 4.5 μg/ml, and to Bel-7402 cells 135.56 $\pm$ 7.62 μg/ml. Under the same conditions, the IC_{50} of vincristine sulfate for injection to HO-8910 cells was 20.7 ± 1.9 μg/ml, and to Bel-7402 cells 25.9 ± 3.4 μg/ml. While at the concentration of 50 μg/ml, compounds **1** and **5** had no effect on the growth of the two cell lines (Table 1). The results indicated that the antitumour activity of compound **4** is very different from that of **5**, although both of them are isoquinoline alkaloids. Compound **4** showed an inhibitery effect to HO-8901 (human ovarian neoplasm) cells almost equivalent to that of vincristine sulfate. The differences may be caused by the cytotoxic activity of the chloride anion.

3. Experimental

3.1. Apparatus

Melting points were determined on a Kofler apparatus and are uncorrected. IR spectra were recorded with a Nicolet NEXUS 670 FT-IR spectrometer. UV spectra were measured on a TU-1901 UV-VIS spectrometer. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. NMR were recorded on a Varian Mercury plus-400 spectrometer. FABMS were measured with a VG-ZAB-HS mass spectrometer, HRESIMS on Bruker APEX II spectrometer. EIMS on HP-5988A GC/MS instrument. Silica gel (200–300 mesh) for CC and silica GF_{254} for TLC were supplied by the Qingdao Marine Chemical factory.

3.2. Plant material

The whole plant of *Carduus crispus* Linn. was collected in Zhang county, Gansu province of China in August 2001. It was identified by Prof. Guo-Liang Zhang, Department of Biology, Lanzhou University. A voucher specimen (No. 20018) was deposited in the Institute of Organic Chemistry, Lanzhou University.

3.3. Extraction and isolation

The air-dried whole plant of *Carduus crispus* L. (5.5 kg) was pulverized and extracted with 95% ethanol three times (7 days each time) at room temperature. The extract was concentrated under reduced pressure and the residue (530 g) was suspended in hot water (60 °C, 1500 ml). This suspension was extracted, successively, with petroleum ether (b.p. 60–90 °C), EtOAc and *n*-BuOH. The EtOAc soluble fraction was concentrated under reduced pressure to yield a residue (11 g). This residue was chromatographed over a silica gel column with a gradient $CHCl_3$–CH_3OH (40 : 1, 20 : 1, 10 : 1, 5 : 1) and CH_3OH as eluent, respectively. Three fractions were collected according to TLC analysis. Fraction 1 (1.4 g) was separated on silica gel column eluted with petroleum ether-EtOAc (10 : 1, 5 : 1), and further purified by a polyamide column eluted with $CHCl_3$–MeOH (20 : 1) to yield **11** (26 mg). Fraction 2 (635 mg) was on chromatographic purifica-

Table 2: ^{13}C NMR and DEPT spectral data of 1, 2 and 3 (DMSO-d_6, δ, ppm, TMS) [a]

C	1	2	3	DEPT	C	1	2	3	DEPT
Aglycone					Inner Glu				
2	164.8	164.8	165.1	C	1	98.9	99.4	98.8	CH
3	104.1	104.0	103.8	CH	2	83.7	83.5	83.6	CH
4	182.1	182.6	182.5	C	3	76.4	76.3	76.3	CH
5	161.7	161.7	161.7	C	4	69.7	69.9	69.7	CH
6	100.2	100.3	100.3	CH	5	77.7	77.7	77.7	CH
7	163.4	163.5	163.4	C	6	61.6	61.3	61.1	CH_2
8	95.6	96.2	96.2	CH	Terminal Glu				
9	157.5	157.4	157.6	C	1	105.3	105.5	105.3	CH
10	106.0	106.0	106.0	C	2	75.5	75.4	75.2	CH
1′	122.0	122.0	121.9	C	3	76.4	76.8	76.8	CH
2′	110.8	110.8	114.2	CH	4	70.3	70.2	70.4	CH
3′	148.7	148.7	146.5	C	5	74.3	77.7	74.2	CH
4′	151.3	151.6	150.7	C	6	64.2	61.2	64.2	CH_2
5′	116.4	116.4	116.7	CH	OAc	171.0		170.9	C
6′	121.1	121.1	119.7	CH		21.2		21.3	CH_3
OMe	56.5	56.5		CH_3					

[a] 100 MHz for **1** and **3**, 75 MHz for **2**

Table 3: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and DEPT spectral data of 4 and 5 (δ, ppm, TMS) [a]

No.	^{1}H		^{13}C	
	4	**5**	**4**	**5**
1	–	–	158.1 (C)	155.9 (C)
3	8.09 (d, J = 6.4)	8.31 (d, J = 6.4)	129.7 (CH)	130.7 (CH)
4	7.67 (d, J = 6.4)	8.10 (d, J = 6.4)	122.6 (CH)	122.1 (CH)
4a	–	–	135.1 (C)	137.0 (C)
5	7.05 (s)	7.65 (s)	106.2 (CH)	107.2 (CH)
6	–	–	156.2 (C)	157.4 (C)
7	–	–	151.8 (C)	152.9 (C)
8	6.96 (s)	7.71 (brs)	105.2 (CH)	105.3 (CH)
8a	–	–	120.6 (C)	122.5 (C)
1′	3.51 (brdd, J = 8.0, 7.6)	3.44 (brdd, J = 8.4, 7.2)	31.4 (CH_2)	28.6 (CH_2)
2′	2.46 (m)	1.96 (m)	20.7 (CH_2)	29.0 (CH_2)
3′	4.72 (brdd, J = 8.8, 6.8)	3.27 (dt, J = 6.4, 6.4)	59.1 (CH_2)	40.1 (CH_2)
–OMe	3.70, 3.71	4.01, 4.02	56.4, 56.6 (CH_3)	57.1, 57.3 (CH_3)
guanidino	–	7.71 (brs), 6.8–7.5 (brs)	–	157.3 (C)

[a] D_2O as solvent for **4** and DMSO-d_6 as solvent for **5**

tion over a silica gel column and eluted with petroleum ether-EtOAc (3 : 1), yielded pure compounds **15** (9 mg) and **10** (86 mg). Fraction 3 (2.2 g) was isolated over a silica gel column, eluting with petroleum ether-Me_2CO (3 : 1), to give compound **9** (42 mg).

The *n*-BuOH soluble fraction (92 g) was chromatographed over a silica gel column and eluted with $CHCl_3$–MeOH (10 : 1, 5 : 1, 3 : 1 and 1 : 1), five fractions were obtained based on TLC. Fraction 1 (5.7 g) was subjected to a silica gel column and eluted with $CHCl_3$–MeOH (10 : 1), a mixture (288 mg) was obtained, then the mixture (62 mg) was purified by PTLC eluting with EtOAc–MeOH (20 : 1) to yield compounds **6** (Rf = 0.67, 32 mg) and **7** (Rf = 0.54, 8 mg). Fraction 2 (6.1 g) was subjected to a silica gel column and eluted with EtOAc–MeOH (20 : 1, 10 : 1) to give three fractions (f_1–f_3). The f_1 (332 mg) was further chromatographed with silica gel column (EtOAc–MeOH, 20 : 1) to yield **8** (38 mg). Fraction f_2 (468 mg) on chromatographic purification over a silica gel column ($CHCl_3$–MeOH–H_2O, 10 : 1 : 0.1) gave **13** (13 mg). Fraction f_3 (580 mg) was rechromatographed on a silica gel column eluting with EtOAc–MeOH (10 : 1) to yield **12** (6 mg) and **14** (18 mg). Fraction 3 (8.3 g) was filtered and the filter residue (4.2 g) was purified over a silica gel column eluting with $CHCl_3$–MeOH (5 : 1) to obtained **5** (1.47 g). Fraction 4 (4.9 g) on chromatographic purification over silica gel column and eluting with $CHCl_3$–MeOH (5 : 1), yielded **4** (48 mg) and **1** (1.54 g). Fraction 5 (26 g) was filtered, the filter residue (3.6 g) was subjected to a silica gel column and eluted with $CHCl_3$–MeOH (5 : 1, 3 : 1). A mixture (1.37 g), was obtained which purified on a polyamide column eluting with H_2O–MeOH (1 : 2, 1 : 4) to yield **3** (46 mg) and **2** (6 mg).

3.3.1. Chrysoeriol 7-O-(2″-O-6‴-O-acetyl-β-D-glucopyranosyl-β-D-glucopyranoside) (**1**)

Yellow amorphous powder. m.p. 208–210 °C; $[\alpha]_D^{18}$ −49.0° (c 0.41, pyridine); UV (MeOH): λ_{max} (logε) = 214 (1.39), 268 (1.12) and 346 (1.51) nm; IR (KBr): ν_{max} = 3381, 3266, 2928, 1749, 1657, 1603, 1566, 1516, 1492, 1434 cm^{-1}; FABMS (Gly): m/z = 667 $[M+H]^+$, 461 $[M+H-Glu-Ac]^+$, 301 $[M+H-2Glu-Ac]^+$; HRESIMS: m/z = 667.1870 $[M+H]^+$ (calcd for $C_{30}H_{33}O_{17}$: 666.1869); ^{1}H NMR (400 MHz, DMSO-d_6,): aglycone: δ = 7.57 (2 H, m, H-2′, 6′), 6.99 (1 H, s, H-3), 6.94 (1 H, dd, J = 8.8 Hz, H-5′), 6.81 (1 H, d, J = 2.4 Hz, H-8), 6.47 (1 H, d, J = 2.4 Hz, H-6), 3.98 (3 H, s, CH_3O–); inner glucose: δ = 5.22 (1 H, d, J = 7.6 Hz, H-1″), 3.50 (3 H, m, H-2″, 5″, 6″a), 3.53 (1 H, t, J = 9.2 Hz, H-3″), 3.24 (1 H, t, J = 9.2 Hz, H-4″), 3.72 (1 H, brd, J = 10.0 Hz, H-6″b); terminal acetylglucose: δ = 4.51 (1 H, d, J = 8.4 Hz, H-1‴), 3.04 (1 H, brt, J = 8.4 Hz, H-2‴), 3.21 (1 H, t, J = 9.2 Hz, H-3‴), 3.08 (1 H, t, J = 9.2 Hz, H-4‴), 3.47 (1 H, m, H-5‴), 3.99 (1 H, dd, J = 10.4, 5.2 Hz, H-6‴a), 4.12 (1 H, brd, J = 10.4 Hz, H-6‴b) and 1.94 (3 H, s, acetyl); ^{13}C NMR and DEPT see Table 2.

3.3.2. Chrysoeriol 7-sophoroside (**2**)

Yellow amorphous powder. $[\alpha]_D^{18}$: −69.8° (c 0.04, pyridine); FABMS (Gly): m/z = 647 $[M+Na]^+$, 631 $[M+Li]^+$; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.57 (2 H, m, H-2′, 6′), 6.97 (1 H, s, H-3), 6.95 (1 H, d, J = 8.8 Hz, H-5′), 6.94 (1 H, d, J = 2.0 Hz, H-8), 6.49 (1 H, d, J = 2.0 Hz, H-6), 5.15 (1 H, d, J = 7.2 Hz, H-1 of innerglucose), 4.45 (1 H, d, J = 7.6 Hz, H-1 of terminal glucose), 3.87 (3 H, s, -OMe); ^{13}C NMR and DEPT see Table 2.

3.3.3. Linariifolioside (**3**)

yellow amorphous powder. $[\alpha]_D^{18}$: −50.6° (c 0.77, pyridine); FABMS (S-Gly): m/z = 675 $[M+Na]^+$ and 659 $[M+Li]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.41 (1 H, d, J = 8.8 Hz, H-6′), 7.40 (1 H, s, H-2′), 6.90 (1 H, d, J = 8.8 Hz, H-5′), 6.75 (1 H, d, J = 2.0 Hz, H-8), 6.72 (1 H, s, H-3), 6.43 (1 H, d, J = 2.0 Hz, H-6), 5.23 (1 H, d, J = 7.2 Hz, H-1 of innerglucose), 4.50 (1 H, d, J = 7.6 Hz, H-1 of terminal glucose), 1.90 (3 H, s, acetyl). ^{13}C NMR and DEPT data see Table 2.

3.3.4. Crispine B (**4**)

White plates. m.p. 206–208 °C; $[\alpha]_D^{18}$: +9.4° (c 0.11, H_2O); UV (MeOH): λ_{max} (log ε) = 237 (0.64) and 308 (0.32) nm; HRESIMS: m/z = 230.1160 $[M-Cl]^+$ (calcd for $C_{14}H_{16}NO_2$: 230.1175); FABMS (Gly): m/z = 231 $[M-Cl+H]^+$, 230 $[M-Cl]^+$; EIMS: m/z = 229, 228, 212, 184, 142, 114; The content of chloride anion: 13.72% (analyzed by silver chloride turbidimetry method); ^{1}H and ^{13}C NMR (DEPT) see Table 3.

3.3.5. Crispine C (**5**)

Pale powder. m.p. 214–216 °C; $[\alpha]_D^{18}$: −3.6° (c 0.84, DMSO); UV (MeOH): λ_{max} (logε) = 230 (1.91), 266 (0.91), 278 (0.83), 312 (1.10) and 325 (1.04) nm; HRESIMS: m/z = 289.1660 $[M+H]^+$ (calcd for $C_{15}H_{21}N_4O_2$: 289.1159); FABMS (Gly): m/z = 289 $[M+H]^+$, 230 $[M-CH_4N_3]^+$; ^{1}H and ^{13}C NMR (DEPT) see Table 3.

3.4. Exhaustive acid hydrolysis

A solution of **1** (ca 5 mg) in 2M HCl (5 ml) was heated to 90 °C in a water bath for 3 h. After cooling, the mixture was extracted with $CHCl_3$. The $CHCl_3$ phase was concentrated to yield an aglycone, which was identified as chrysoeriol on the basis of ^{1}H NMR and TLC comparison with an authentic sample. The aqueous layer was concentrated under reduced pressure. The glucose was detected by direct comparison with an authentic sample (PC, EtOAc-pyridine-H_2O, 12 : 5 : 4).

3.5. Antitumour assay

The antitumour activity of all tested compounds was determined in 96-well microtiter plates by the sulforhodamine B method (Skehan et al, 1990). Human ovarian neoplasm cell HO-8910 and human hepatoma cell Bel-7402 were routinely maintained in RPMI-1640 medium supplemented with 10% inactivated calf serum at 37 °C in the presence of 5% CO_2. Exponentially growing cells were used for the experiment. The percentage survival rates of cells exposed to the compounds were calculated by assuming the survival rate of untreated cells to be 100%. The 50% inhibition concentration (IC_{50}) was calculated by the linear regression of concentration and cell viability.

Acknowledgement: This project was supported by the National Nature Science Foundation of China (No.2997201).

References

Chen YZ, Zhang HD, Zhang SM et al. (1989) Studies on the chemical constituents of *Lagotis brachystachya* Maxim Chem J Chin Univ 10: 260–262.

Chenon MT, Pugmire RJ, Grant DM et al. (1975) Carbon-13 magnetic resonance. XXV. A basic set of parameters from the investigation of tautomerism in purines established from carbon-13 magnetic resonance studies using certain purines and pyrrolo[2,3-d]pyrimidines. J Am Chem Soc. 97: 4627–4636.

Deletis Flora Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita (1979) Flora Republicae popularis Sinicae, Institutum Academiae Sience Press, Beijing, 78: p. 157–158.

Jiangsu New Medical College (1977) Chinese Medicine Dictionary, Shanghai People's Publishing House. Shanghai, p. 276–277.

Lee KH, Tagahara K, Suzaki H et al. (1981) Antitumor angents 49. tricin, kaempferol-3-*O*-β-D-glucopyranoside and (+)-nor tracheenin, antileukemic principles from *Wikstroemia indica*. J Nat Prod 44: 530–435.

Ma CY, Zhu KX, Yang DM et al. (1991) Studies on chemical constituents of *Veronica linariifolia* Pall ex link. sub. *dilatata* (nakai et kitagawa) Hong. Acta Pharm Sinica 26: 203–208.

Markham KR, Ternai B, Stanley R et al. (1978) Carbon-13 NMR studies of flavonoids – III. Tetrahedron 34: 1389–1397.

Markham KR, Vilain C, Molly BPG (1985) Uniformity and distinctness of *Phyllocladus* as evidenced by flavonoid accumulation. Phytochemistry 24: 2607–2609.

Matthes HWD, Luu B, Ourisson G (1980) Cytotoxic compounds of *Zingiber zerumbet, Curcuma zedoaria* and *C. domestica.* Phytochemistry 19: 2643–2650.

Niwa M, Iwadare Y, Wu YC et al. (1988) The new phenylpropanoid glycosides from *Wikstroemia sikokiana.* Chem Pharm Bull 36: 1158–1161.

Sadtler Research Laboratories, Inc. (1969); Sadtler Standard N.M.R. Spectra 7–9: 4407 m.

Shen CC, Chang YS, Ho LK (1993) Nuclear magnetic resonance studies of 5,7-dihydroxyflavonoid. Phytochemistry 34: 843–845.

Skehan P, Storeny R, Scudiero D (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 82: 1107–1109.

Thorpe MC, Cobarn WC, Montgomery JA (1974) The ^{13}C nuclear magnetic resonance spectra of some 2-,6- and 2,6-substituted purines. J Magn Reson 15: 98–112.

Umber RE (1980) The flavonoid chemistry of *Glandularia bipinnatifida* (Verbenaceae) and allied species. Am J Bot 67: 935–941.

Wagner H, Chari VM (1976) ^{13}C-NMR Spektren natürlich vorkommender Flavonoide. Tetrahedron Let 21: 1799–1802.

Zhang QZ, Tu GZ, Zhao YY et al. (2002) Novel bioactive isoquinoline alkaloids from *Carduus crispus*. Tetrahedron 58: 6795–6798.