Anthrone *C*-Glucosides from *Rheum emodi*

Liselotte KRENN,*[,a] Riddhi PRADHAN,[a] Armin PRESSER,[b] Gottfried REZNICEK,[a] and Brigitte KOPP[a]

[a] *Institute of Pharmacognosy, University of Vienna, Pharmacy-Center; Althanstrasse 14, A-1090 Vienna, Austria: and* [b] *Institute of Pharmaceutical Chemistry, Karl-Franzens-University Graz; Universitätsplatz 1, A-8010 Graz, Austria.*
Received October 9, 2003; accepted January 21, 2004

In a study of the anthraderivatives in roots of *Rheum emodi*, three new anthrone *C*-glucosides, named 10-hydroxycascaroside C (1), 10-hydroxycascaroside D (2) and 10*R*-chrysaloin 1-*O*-β-D-glucopyranoside (3) were isolated besides the rare compounds cascaroside C (4), cascaroside D (5) and cassialoin (6). Additionally the investigation resulted in the isolation of an acetylated chrysophanol glucoside, 8-*O*-β-D-(6′-*O*-acetyl)glucopyranosylchrysophanol (7). The structures were established by comprehensive spectroscopic investigations.

Key words *Rheum emodi*; anthrone-glycoside; cascaroside; cassialoin; ayurvedic medicine

The roots of *Rheum emodi* WALL. (syn. *Rheum australe* D. DON, Polygonaceae) are widely used in Ayurvedic and Asian folk medicine. The drug is administered as a stomachic, purgative, astringent, tonic, in biliousness, lumbago, piles, chronic bronchitis, and asthma as well as in certain skin diseases.[1—3] In previous papers mainly anthraquinones like emodin, chrysophanol, physcion, their respective 8-*O*-glucosides and 8-*O*-gentiobiosides and rhein, the flavonoid rutin and torachrysone 8-*O*-β-D-glucoside were identified.[4—6] Additionally rheinal, rhein-11-*O*-β-D-glucoside, revandchinones 1, 2, 3 and 4 as well as very recently the first sulfated anthraquinone glycoside—sulfemodin 8-*O*-β-D-glucoside—and rare auronols—carpusin and maesopsin—have been reported.[6—8] In this continuous study on anthrone glycosides, the isolation and structure elucidation of three new compounds, besides three known anthrone *C*-glycosides as well as an acetylated anthraquinone glucoside is described.

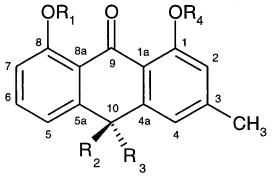
Results and Discussion

Dried, pulverized roots of *R. emodi* were pre-extracted with petroleum ether for the removal of non-polar compounds and then extracted with methanol. The residue of the methanolic extract was fractionated by vacuum liquid chromatography (VLC) and column chromatography (CC) with conventional stationary and mobile phases. Final purification of several fractions by semipreparative HPLC resulted in the isolation of seven compounds.

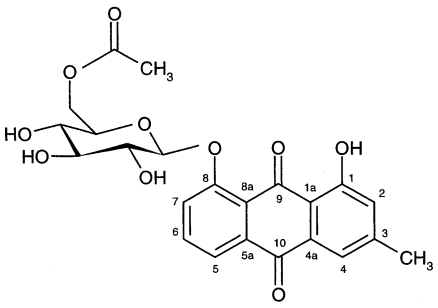
Compound **1** was obtained as pale yellow amorphous substance. The IR spectrum displayed bands of hydroxyl group at 3392 cm^{-1}, chelated carbonyl group at 1634 cm^{-1} and aromatic ring at 1573 cm^{-1}. From the high resolution electrospray ionisation mass spectrometry (HR-ESI-MS), a molecular weight of 580 in accordance with the molecular formula $C_{27}H_{32}O_{14}$ was deduced. In the ^{1}H-NMR spectrum five aromatic protons were shown between δ 6.76—7.77. The broad singlets at δ 6.76 and δ 7.20 were assigned to H-2 and H-4, the triplet at δ 7.64 to H-6 and the two double doublets at δ 7.77 and δ 7.46 to H-5 and H-7, respectively. The shifts proved an anthrone skeleton with a hydroxy group attached to C-10. An aromatic methyl group was observed at δ 2.41. The attachment of this group in position C-3 was shown by the correlation of the methyl protons with C-2, C-3 and C-4 in the heteronuclear multiple-bond correlation (HMBC) spectrum. From the resonances of the anomeric protons of two hexoses one *O*-glycosidic and one *C*-glycosidic attachment was deduced. The correlated spectroscopy (COSY) experiment allowed the unequivocal assignment of the sugar protons. By the ^{13}C-NMR data a 10-hydroxy-chrysophanol-anthrone skeleton was confirmed. The chemical shifts of the carbohydrate carbons followed the usual pattern for a *C*- and an *O*-glucopyranoside. Due to the downfield shifts of H-5 and H-7 as well as of C-5 and C-7 in accordance with the nuclear Overhauser effect (NOE) of H-7 at H-1″ and the cross peak of the H-1″ to C-8 in the HMBC spectrum the 8-*O*-glucopyranoside was proven. The unambiguous assignment of the ^{1}H- and ^{13}C-resonances was performed by combining the information obtained by several two-dimensional NMR-techniques (COSY, heteronuclear single quantum coherence (HSQC), heteronuclear single quantum coherence-total correlation spectroscopy (HSQC-TOCSY), HMBC).[9] The configuration at C-10 was deduced by a combination of NMR and energy minimum calculations: Investigations by Manitto *et al.*[10,11] concerning the conformational stability of cascarosides and aloins demonstrated that the absolute configuration at C-10 can be inferred from NOE correlations. Energy minimum computations of **1** using the MM2 force field showed that a change from a H- to an OH-group at C-10 does not have an influence on the preferred conformation of the molecule. This observation as well as a positive NOE of H-5 with H-1′ and H-4 with H-2′ and the aromatic methyl group were indicative for the configuration comparable to cascaroside C (**4**).[11] Thus, **1** was identified as 10-hydroxycascaroside C.

From the IR spectrum of **2**, similar to **1**, the presence of hydroxyl group (3393 cm^{-1}), carbonyl group (1636 cm^{-1}) and aromatic ring (1573 cm^{-1}) was deduced. Its molecular formula of $C_{27}H_{32}O_{14}$ was determined on the basis of HR-ESI-MS data. The ^{1}H-NMR spectrum showed five aromatic protons, the proton of a vinylic methyl group and fourteen sugar protons. By COSY the unequivocal assignment of the sugar protons of an *O*-glycosidic and a *C*-glycosidic moiety was possible, which due to the ^{13}C-NMR shifts were identified as glucopyranosides. From the presence of only one carbonyl-carbon the anthrone structure of the aglycone was deduced. The shift of C-10 at δ 77.1 proved a hydroxyl function in this position. In analogy to **1**, HMBC and NOE experiments revealed the attachment of the *O*-glycosidic sugar at C-8 and the substitution pattern of the skeleton. In the CD spectra **1** and **2** showed almost opposite Cotton effects at 212 nm, indicating that **1** and **2** differ only in the configuration at C-10.[12] On the basis of these data and the observed

* To whom correspondence should be addressed. e-mail: liselotte.krenn@univie.ac.at



	R_1	R_2	R_3	R_4
1	Glc	Glc	OH	H
2	Glc	OH	Glc	H
3	H	H	Glc	Glc
4	Glc	Glc	H	H
5	Glc	H	Glc	H
6	H	OH	Glc	H



7

Fig. 1. Structures of Compounds **1**—**7**

NOE's (correlation of H-4 with H-1′ and the aromatic methyl group) the structure of **2** was unambiguously elucidated as 10-hydroxycascaroside D.

The IR absorption bands of **3** at 3369, 1628 and 1567 cm^{-1} indicated the presence of chelated hydroxyl group, chelated carbonyl group and aromatic ring. Based on HR-ESI-MS data a molecular weight of 564 with the molecular formula $C_{27}H_{32}O_{13}$ was deduced. The ^{1}H- and ^{13}C-NMR spectra indicated **3** to be an anthrone glycoside due to the characteristic resonances at δ 4.56 and δ 46.3 (H-10 and C-10, respectively). The signals at δ 3.35 and δ 85.5 were typical for the C-1′ anomeric position of a *C*-glycoside and those at δ 4.95 and δ 105.3 for the C-1″ anomeric position of an *O*-glycoside. The ^{1}H-NMR spectrum revealed five aromatic protons in the anthrone nucleus and an aromatic methyl group. The HMBC experiments showed cross-peaks between the methyl group and the aromatic protons H-2 and H-4, thus proving the methylation of C-3. By COSY, HSQC and HMBC experiments the unambiguous assignment of the sugar signals was possible, which were in good correlation with those of cascaroside E and F.[13] The CD spectrum of **3** was largely comparable to the one of aloin B.[10] The connection of the *O*-glucose as well as the absolute configuration at C-10 were unequivocally deduced from selective NOE experiments according to refs. 10, 11: irradiation at the resonance of H-2 gave a positive NOE at the signal of H-1″, H-5 showed a correlation with H-2′ and H-4 with H-1′. Thus **3** was identified as 10*R*-chrysaloin 1-*O*-β-D-glucopyranoside.

In addition, three further, rare anthrone glycosides and an acetylated anthraquinone glycoside were isolated. The occurrence of cascaroside C (**4**) and cascaroside D (**5**) until now was limited to *Rhamnus purshianus* DC. only.[14] This is the first report of **4** and **5** in another species than *Rhamnus*. Cassialoin (**6**) is also quite rare in nature and was previously proven in *Cassia garretiana* Craib. only.[15] 8-*O*-β-D-(6′-*O*-acetyl)glucopyranosyl-chrysophanol (**7**) was isolated for the first time from *Rheum palmatum* L. recently.[16]

Experimental

General Procedures IR spectra were determined as film spectra on silicium disks on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. NMR spectra were recorded on a Varian Unity Inova 400 MHz NMR spectrometer in 5 mm sample tubes with tetramethylsilane as internal standard. COSY, HSQC, HMBC, HSQC-TOCSY, and selective NOE experiments were obtained using conventional pulse sequences. HMBC experiments were optimized for a longe-range coupling constant of 8 Hz. FAB-MS were recorded on a Finnigan MAT 95 A in the negative- and positive-ion modes, with an acceleration voltage of 5.0 kV in a glycerol matrix. ESI-MS were recorded on a PE Sciex API 150 EX single quadrupole instrument configurated for negative ionisation, the orifice plate voltage set at −20 and −80 V. Full scan spectra were acquired over the range 200—700 *mz*. Scan time: 2 s. HR-ESI-MS were recorded on a Biosystems QStar (Q-TOF) configurated for ESI negative mode. Semipreparative HPLC was carried out on two ISCO 2350 HPLC pumps with a Linear UVIS-205 absorbance detector on a Nucleosil 100-7 C_{18}, 20×250 mm column. Silica DCC for VLC, Sephadex-LH-20 and silica gel 60 for CC were obtained from ICN Pharmaceuticals (Eschwege, Germany), Pharmacia Biotech (Uppsala, Sweden), and Merck (Darmstadt, Germany), respectively. TLC was performed on precoated plates (silica gel 60 F_{254}, RP-2 F_{254}, RP-18 F_{254}, Merck, Germany) with the systems A: toluene–ethyl formate–formic acid (75 : 24 : 1), B: $CHCl_3$–MeOH–H_2O (90 : 5 : 5), C: $CHCl_3$–MeOH–H_2O (75 : 20 : 2), D: $CHCl_3$–MeOH–H_2O (65 : 30 : 6), E: MeOH–H_2O (1 : 1), F: MeOH. After drying, the plates were examined under UV_{366} light.

Plant Material Roots of *Rheum emodi* were collected from Gorkha district, Nepal in October 1996 and authentified by Senior Botanist T. M. Shresta, Department of Natural Resources, Ministry of Forest and Soil Conservation, His Majesty's Government, Kathmandu, Nepal. A specimen (No. Rhemsa 6/96) is deposited in the Institute of Pharmacognosy, University of Vienna.

Extraction and Isolation The air-dried, powdered roots (600 g, 3.6% total anthraquinone content, determined according to the monograph "Rhubarb" in the European Pharmacopoeia) were pre-extracted three times in portions of 150 g with petroleum ether (1.5 l) under reflux. The remaining drug was dried and then extracted four times with MeOH (1.5 l) under the same conditions. The extraction yielded 8 g petroleum ether extract (40% total anthraquinone content) and 200 g MeOH extract (9.1% total anthraquinone content). The latter was fractionated by VLC on silica DCC 60 (54×12.5 cm) using $CHCl_3$–MeOH-mixtures of increasing polarity to yield twelve fractions (VM 1—VM 12). A fraction of high polarity (VM 10; 58.4 g) was separated by VLC (70×8.2 cm) on silica DCC 60 with EtOAc–MeOH–H_2O mixtures as eluent to yield 7 fractions. Fractions 3 (14.1 g) and 5 (16.3 g) were again submitted to VLC (both columns 60×4.6 cm) on silica DCC 60. The elution with $CHCl_3$–MeOH–H_2O mixtures resulted in 14 (VLC I) and 16 (VLC II) subfractions, respectively. Subfraction VLC I-7 (2.05 g) and subfraction VLC II-12 (4.43 g) were further separated by CC (CC I 80×2 cm; CC II 100×4 cm) on Sephadex LH 20. The elution was performed with MeOH–H_2O mixtures of decreasing polarity. CC I yielded 9 fractions and CC II 12 fractions. By semipreparative HPLC under elution with MeOH–H_2O from fraction CC I-7 (290 mg) **1** (100 mg), **3** (8 mg) and **4** (10 mg) and from fraction CC II-5 (590 mg) **2** (65 mg) and **5** (30 mg) were isolated. The fraction (VM 4; 2.3 g) was separated by CC (100×3 cm) on silica gel 60 with EtOAc–MeOH–H_2O mixtures of increasing polarity, yielding 8 subfractions. By CC (80×3 cm) of subfractions 3 and 4 (660 mg) on Sephadex LH 20 with MeOH–H_2O mixtures of decreasing polarity 290 mg of a fraction enriched in **6** were obtained. After final purification by CC on silica gel 60 **6** (92 mg) was isolated. VLC (60×4.6 cm) on silica DCC 60 of fraction VM 9 (24.3 g) using EtOAc–MeOH–H_2O mixtures as mobile phase resulted in 7 subfractions. From subfraction 5 (2.9 g) by rechromatography on the same stationary phase (VLC; 40×4 cm) with $CHCl_3$–MeOH–H_2O mixtures **7** (53 mg) was obtained.

10-Hydroxycascaroside C (**1**): Pale yellow amorphous. $[\alpha]_D^{21}$ −41.2° (c=0.051, MeOH). IR ν_{max} silicium disk cm^{-1}: 3392, 1634, 1573. UV λ_{max} (MeOH) nm (log ε): 266 (3.75), 301 (3.89), 322 sh (3.83). ESI-MS *m/z* 579 $[M-H]^-$. HR-MS *m/z*: 579.1731 $[M-H]^-$ (Calcd for $C_{27}H_{31}O_{14}$: 579.1714).

CD (c=5.72×10^{-5}, MeOH) $\Delta\varepsilon^{21}$ (nm): −15.37 (211). ^{1}H-NMR (400 MHz, CD_3OD) δ: 2.41 (3H, s, CH_3), 2.80 (1H, t, J=9.6 Hz, H-4′), 2.85—2.91 (1H, m, H-5′), 3.00 (1H, t, J=9.2 Hz, H-2′), 3.19 (1H, d, J=9.6 Hz, H-1′), 3.28 (1H, t, J=8.8 Hz, H-3′), 3.32 (1H, dd, J=11.5, 5.7 Hz, H-6′$_a$), 3.43 (1H, t, J=8.8 Hz, H-4″), 3.44—3.50 (1H, m, H-5″), 3.52 (1H, t, J=9.2 Hz, H-3″), 3.53 (1H, d, J=11.5 Hz, H-6′$_b$), 3.62 (1H, dd, J=9.2, 7.6 Hz, H-2″), 3.73 (1H, dd, J=12.1, 5.5 Hz, H-6″$_a$), 3.92 (1H, dd, J=12.1, 2.2 Hz, H-6″$_b$), 4.93 (1H, d, J=7.6 Hz, H-1″), 6.76 (1H, br s, H-2), 7.20 (1H, br s, H-4), 7.46 (1H, dd, J=8.0, 1.2 Hz, H-7), 7.64 (1H, t, J=8.0 Hz, H-6), 7.77 (1H, dd, J=8.0, 1.2 Hz, H-5). ^{13}C-NMR (100 MHz, CD_3OD) δ: 22.5 (CH_3), 62.8 (C-6″), 63.5 (C-6′), 71.5 (C-4″), 71.9 (C-4′), 73.3 (C-2′), 75.3 (C-2″), 77.2 (C-10), 77.5 (C-3″), 78.9 (C-5″), 79.7 (C-3′), 81.8 (C-5′), 84.0 (C-1′), 105.5 (C-1″), 118.0 (C-1a), 118.3 (C-2), 119.5 (C-4), 120.0 (C-7), 121.3 (C-5), 124.1 (C-8a), 136.1 (C-6), 145.0 (C-4a), 147.5 (C-3), 149.8 (C-5a), 158.9 (C-8), 162.1 (C-1), 191.6 (C-9).

10-Hydroxycascaroside D (**2**): Pale yellow amorphous. $[\alpha]_D^{21}$ −81.9° (c=0.085, MeOH). IR ν_{max} silicium disk cm^{-1}: 3393, 1636, 1573. UV λ_{max} (MeOH) nm (log ε): 263 (3.73), 298 (3.91), 325 sh (3.86). CD (c=5.21×10^{-5}, MeOH) $\Delta\varepsilon^{21}$ (nm): +14.99 (212). ESI-MS m/z 579 [M−H]$^-$. HR-MS m/z: 579.1725 [M−H]$^-$ (Calcd for $C_{27}H_{31}O_{14}$: 579.1714). ^{1}H-NMR (400 MHz, CD_3OD) δ: 2.36 (3H, s, CH_3), 2.82—2.93 (2H, m, H-4′, H-5′), 3.01 (1H, t, J=9.6 Hz, H-2′), 3.19 (1H, d, J=9.6 Hz, H-1′), 3.27 (1H, t, J=8.5 Hz, H-3′), 3.37 (1H, dd, J=11.8, 4.9 Hz, H-6′$_a$), 3.41—3.49 (1H, m, H-4″), 3.44—3.50 (1H, m, H-3″), 3.45—3.51 (1H, m, H-5″), 3.52 (1H, d, J=11.8 Hz, H-6′$_b$), 3.65 (1H, t, J=7.8 Hz, H-2″), 3.73 (1H, dd, J=11.9, 5.0 Hz, H-6″$_a$), 3.92 (1H, d, J=11.9 Hz, H-6″$_b$), 4.93 (1H, d, J=7.7 Hz, H-1″), 6.71 (1H, br s, H-2), 7.25 (1H, br s, H-4), 7.45 (1H, dd, J=8.0, 1.2 Hz, H-7), 7.61—7.65 (2H, m, H-5, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δ: 22.5 (CH_3), 62.8 (C-6″), 63.2 (C-6′), 71.5 (C-4′, C-4″), 73.5 (C-2′), 75.0 (C-2″), 77.1 (C-10), 78.1 (C-3″), 78.7 (C-5″), 79.6 (C-3′), 81.6 (C-5′), 84.5 (C-1′), 103.7 (C-1″), 117.8 (C-1a), 118.0 (C-7), 118.1 (C-4), 118.3 (C-2), 121.9 (C-5), 124.0 (C-8a), 135.0 (C-6), 147.3 (C-3), 147.7 (C-5a), 147.9 (C-4a), 159.0 (C-8), 161.7 (C-1), 191.2 (C-9).

10*R*-Chrysaloin 1-*O*-β-D-glucopyranoside (**3**): Pale yellow amorphous. $[\alpha]_D^{21}$ −56.6° (c=0.049, MeOH). IR ν_{max} silicium disk cm^{-1}: 3369, 1628, 1567. UV λ_{max} (MeOH) nm (log ε): 249 (3.78), 294 (3.91), 321 (3.86). CD (c=5.85×10^{-5}, MeOH) $\Delta\varepsilon^{21}$ (nm): +7.10 (352), −13.68 (297), +5.39 (258), +5.30 (242). ESI-MS m/z 563 [M−H]$^-$. HR-MS m/z: 563.1740 [M−H]$^-$ (Calcd for $C_{27}H_{31}O_{13}$: 563.1765). ^{1}H-NMR (400 MHz, CD_3OD) δ: 2.43 (3H, s, CH_3), 2.83 (1H, t, J=9.6 Hz, H-4′), 2.85—2.91 (1H, m, H-5′), 3.00 (1H, t, J=9.2 Hz, H-2′), 3.28 (1H, t, J=8.8 Hz, H-3′), 3.34 (1H, dd, J=11.5, 4.7 Hz, H-6′$_a$), 3.35 (1H, d, J=9.7 Hz, H-1′), 3.41 (1H, t, J=9.3 Hz, H-4″), 3.47—3.52 (1H, m, H-5″), 3.52 (1H, t, J=9.2 Hz, H-3″), 3.53 (1H, d, J=11.5 Hz, H-6′$_b$), 3.62 (1H, dd, J=9.2, 7.6 Hz, H-2″), 3.72 (1H, dd, J=12.2, 5.8 Hz, H-6″$_a$), 3.94 (1H, dd, J=12.2, 2.2 Hz, H-6″$_b$), 4.56 (1H, s, H-10), 4.95 (1H, d, J=7.6 Hz, H-1″), 6.85 (1H, d, J=8.0 Hz, H-7), 7.05 (1H, d, J=8.0 Hz, H-5), 7.15 (1H, br s, H-4), 7.25 (1H, br s, H-2), 7.43 (1H, t, J=7.8 Hz, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δ: 22.2 (CH_3), 46.3 (C-10), 62.9 (C-6″), 63.7 (C-6′), 71.7 (C-4″), 72.2 (C-2′), 72.4 (C-4′), 75.3 (C-2″), 77.6 (C-3″), 78.9 (C-5″), 80.2 (C-3′), 81.8 (C-5′), 85.5 (C-1′), 105.3 (C-1″), 117.3 (C-7), 119.5 (C-2), 121.0 (C-5), 122.0 (C-8a), 123.0 (C-1a), 124.6 (C-4), 135.6 (C-6), 141.5 (C-5a), 147.7 (C-4a), 148.0 (C-3), 159.6 (C-1), 162.0 (C-8), 193.0 (C-9).

Acknowledgements The authors are grateful to Dr. K. K. Mayer, Faculty of Natural Sciences IV, University of Regensburg, Germany, for FAB-MS measurements.

References

1) Dobremez J. F., "Mountain, Environment and Development," ed. by Swiss Association of Technical Assistance in Nepal, Sahyogi Press, Kathmandu, 1982.
2) Coburn B., *Kailash*, **11**, 55—88 (1984).
3) Kapoor L. D., "Handbook of Ayurvedic Medicinal Plants," CRC Press, Boca Raton, 1990, p. 487.
4) Prasad P., Purohit M. C., *Curr. Sci.*, **80**, 734—736 (2001).
5) Agrawal S. K., Singh S. S., Verma S., Kumar S., *J. Ethnopharmacol.*, **72**, 43—46 (2000).
6) Krenn L., Presser A., Pradhan R., Bahr B., Paper D. H., Mayer K. K., Kopp B., *J. Nat. Prod.*, **66**, 1107—1109 (2003).
7) Agrawal S. K., Singh S. S., Verma S., Kumar S., *Indian J. Chem. B*, **38B**, 749—751 (1999).
8) Suresh Babu K., Srinivas P. V., Praveen B., Hara Kishore K., Suryanarayana Murty U., Madhusudana Rao J., *Phytochemistry*, **62**, 203—207 (2003).
9) Timothy C., "High-resolution NMR Techniques in Organic Chemistry," Pergamon Press, New York, 1999.
10) Manitto P., Monti D., Speranza G., *J. Chem. Soc.*, *Perkin Trans. 1*, **1990**, 1297—1300 (1990).
11) Manitto P., Monti D., Speranza G., Mulinacci N., Vincieri F. F., Griffini A., Pifferi G., *J. Chem. Soc.*, *Perkin Trans. 1*, **1993**, 1577—1580 (1993).
12) Yamagishi T., Nishizawa M., Ikura M., Hikichi K., Nonaka G.-I., Nishioka I., *Chem. Pharm. Bull.*, **35**, 3132—3138 (1987).
13) Manitto P., Monti D., Speranza G., *J. Nat. Prod.*, **58**, 419—423 (1995).
14) Wagner H., Demuth G., *Zeitschrift f. Naturforsch. B*, **31b**, 267—272 (1976).
15) Hata K., Baba K., Kozawa M., *Chem. Pharm. Bull.*, **26**, 3792—3797 (1978).
16) Shi Y.-Q., Fukai T., Sakagami H., Kuroda J., Miyaoka R., Tamura M., Yoshida N., Nomura T., *Anticancer Res.*, **21**, 2847—2854 (2001).