Turkish Journal of Chemistry

Volume 30 | Number 1 Article 2

1-1-2006

Phytochemical Studies on the Underground Parts of Asperula taurina subsp. caucasica

UFUK ÖZGEN

CAVİT KAZAZ

HASAN SEÇEN

MAKSUT COŞKUN

Follow this and additional works at: https://journals.tubitak.gov.tr/chem

 Part of the Chemistry Commons

Recommended Citation

ÖZGEN, UFUK; KAZAZ, CAVİT; SEÇEN, HASAN; and COŞKUN, MAKSUT (2006) "Phytochemical Studies on the Underground Parts of Asperula taurina subsp. caucasica," *Turkish Journal of Chemistry*: Vol. 30: No. 1, Article 2. Available at: https://journals.tubitak.gov.tr/chem/vol30/iss1/2

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Chemistry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Turk J Chem
30 (2006) , 15 – 20.
© TÜBİTAK

Phytochemical Studies on the Underground Parts of *Asperula taurina* subsp. *caucasica*

Ufuk ÖZGEN[1*], Cavit KAZAZ[2], Hasan SEÇEN[2], Maksut COŞKUN[3]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Atatürk University, 25240 Erzurum- TURKEY*
e-mail: uozgen@atauni.edu.tr
[2]*Department of Chemistry, Faculty of Arts and Science, Atatürk University, 25240 Erzurum-TURKEY*
[3]*Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University, 06100 Tandoğan, Ankara-TURKEY*

Received 10.03.2005

One naphthohydroquinone (mollugin) (**1**), 3 anthraquinones (1-hydroxy-2-methyl-9,10-anthraqinone (**2**), 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone (**4**) and 1,3-dihydroxy-2-carboxy-9,10-anthraquinone (**7,** munjistin)), β-sitosterol (**3**), 1 naphthalene glycoside (2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone, 1,4-di-O-β-glucoside (**5**)) and 1 anthraquinone glycoside (lucidin-3-O-β-primeveroside (**6**)) were isolated from the underground parts of *A. taurina* subsp. *caucasica*. The structures of the isolates were established by MS, ^{1}H-NMR and ^{13}C-NMR analysis.

Key Words: *Asperula taurina* subsp. *caucasica,* Rubiaceae, anthraquinone, anthraquinone glycoside, naphthohydroquinone, naphthalene glycoside.

Introduction

The family Rubiaceae is represented by about 500 genera and 6000 species, most of them tropical trees and shrubs[1]. Some species belonging to this family contain quinonic compounds (anthraquinones, naphthoquinones, naphthohydroquinones and their glycosides)[2−5], iridoids[6], coumarins[7], triterpenes[8] and flavonoids[9]. The subterranean parts of some genera belonging to Rubiaceae are rich in quinonic compounds. *Rubia, Galium, Asperula* and *Morinda* species contain quinonic compounds[2−5]. Some 9,10-anthraquinones and their glycosides were isolated from the underground parts of *Asperula odorata* and *A. besseriana*[2,10].

The genus *Asperula* has about 200 known species[1]. This genus has 39 species in Turkey, and 26 taxa belonging to these species are endemic. *Asperula taurina* subsp. *caucasica* grows in northeast Turkey[11]. A survey of the literature revealed that there have been no phytochemical studies dealing with *A. taurina*. We herein report the isolation and characterization of some different structural compounds from *A. taurina* subsp. *caucasica*.

*Corresponding author

Experimental

General: NMR spectra were recorded on a Varian Mercury 400 MHz NMR spectrometer and 270.05 (67.80) JEOL NMR spectrometer. EI-MS spectra were recorded on a Thermo-Finnigan and JEOL JMS D300 mass spectrometer. Column chromatography was performed on silica gel 60 (0.063-0.200 μ, Merck), RP-18 (LiChroprep®, 25-40 μ, Merck) and Sephadex LH-20 (Sigma-Aldrich). Preparative TLC was performed with silica gel F_{254} plates (20 x 20 cm, 0.5 mm, Merck).

Plant Material: The underground parts (roots and rhizomes) of *A. taurina* L. subsp. *caucasica* (Pobed.) Ehrend. (Syn.: *A. caucasica* Pobed.) were collected from Ormanüstü village (625 m) (Maçka district, Trabzon province, Turkey) in August 2000. It was identified by Dr. Ufuk Özgen. A voucher specimen (AEF 19791) is deposited at the Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu (AEF).

Extraction and Isolation: The air-dried and powdered underground parts (roots and rhizomes) (700 g) of *A. taurina* subsp. *caucasica* were extracted with methanol (3000 mL x 3) under reflux for 3 h for each extraction at 40 °C. The combined methanolic extracts were evaporated to dryness (73 g, yield 10.4%) under reduced pressure at 40 °C. Methanol extract was suspended with 300 mL of water:methanol (9:1). This mixture was partitioned against chloroform (300 mL x 3). Chloroform fractions were combined and evaporated at reduced pressure at 40 °C. The chloroform extract was 14 g. The aqueous fraction was evaporated to give a residue (59 g).

The chloroform fraction (12 g) was subjected to silica gel column chromatography. Elution was performed with an n-hexane-ethyl acetate mixture with gradient elution. Similar fractions determined by TLC were combined. Mollugin (**1**, 300 mg), 1-hydroxy-2-methyl-9,10-anthraquinone (**2**, 10 mg), β-Sitosterol (**3**, 50 mg), and 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone (**4**, 15 mg) were obtained. Column chromatography, preparative TLC and recrystallization were used to obtain pure compounds.

The aqueous extract (25 g) was subjected to a Sephadex LH-20 column. Elution was performed with methanol. Six fractions were collected. A white powder was obtained from the third fraction (800 mg). It was subjected to a silica gel column ($CHCl_3$:MeOH:water 70:30:3, v/v/v) and then an RP-18 silica gel column (MeOH:H_2O, 1:1, v/v). 2-Carbomethoxy-3-prenyl-1,4-naphtho-hydroquinone, 1,4-di-O-β-glucoside (**5**, 20 mg) was obtained. Fraction 4 (600 mg) gave a yellow powder. It was purified using water on a Sephadex column and lucidin-3-O-β-primeveroside was obtained (**6**, 20 mg). Fraction 5 (100 mg) was subjected to a Sephadex column using MeOH and 1,3-dihydroxy-2-carboxy-9,10-anthraquinone (**7,** munjistin) (8 mg) was obtained.

Mollugin (6-hydroxy-2,2-dimethyl-2H-naphtho[1,2-b]pyran-5-carboxylic acid methyl ester) (1): Yellow crystal; **EI-MS (m/e)** 284 [M^+] (33%), 269 (21%), 252 (39%), 237 (100%); **^{1}H-NMR** (270 MHz, $CDCl_3$): δ 12.16 (s, 1H, OH), 8.38 (bd, 1H, H-7 or H-10, J = 8.3 Hz), 8.18 (bd, 1H, H-7 or H-10, J = 8.3 Hz), 7.61 (ddd, 1H, H-8 or H-9, J = 8.3, J = 6.9, J = 1.3 Hz), 7.51 (ddd, 1H, H-8 or H-9, J = 8.3, J = 6.9, J = 1.3 Hz), 7.12 (d, 1H, H-4, J = 9.9 Hz), 5.68 (d, 1H, H-3, J = 9.9 Hz), 4.01 (s, 3H, OCH_3), 1.48 (s, 6H, 2xCH_3); **^{13}C-NMR** (67.8 MHz, $CDCl_3$): δ 172.5 (s), 156.5 (s), 141.6 (s), 129.4 (d), 129.1 (s), 128.8 (d), 126.5 (d), 125.1 (s), 124.0 (d), 122.3 (d), 121.9 (d), 112.6 (s), 102.2 (s), 74.6 (s), 52.3 (q), 26.8 (q). EI-MS, ^{1}H-NMR and ^{13}C-NMR data agree with the literature[12–14].

1-Hydroxy-2-methyl-9,10-anthraquinone (2): Yellow crystal; **EI-MS (m/e)** 238 [M^+] (100%), 209 (14%), 181 (22%), 152 (23%), 76 (12%); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 8.34-8.29 (m, 2H, H-5 and

H-8), 7.82-7.80 (m, 2H, H-6 and H-7), 7.77 (d, 1H, H-3 or H-4, $J = 7.7$ Hz), 7.55 (d, 1H, H-3 or H-4, $J = 7.7$ Hz), 2.39 (s, 3H, CH_3). EI-MS and ^{1}H-NMR are in good agreement with the data given in the literature[15].

β-Sitosterol (5-Stigmasten-3β-ol) (3): White crystal; **EI-MS (m/e)** 414 [M^+] (100%), 396 (54%), 381 (21%); **^{1}H-NMR** (270 MHz, $CDCl_3$) (selected data): δ 5.34 (m, 1H, H-6), 3.51 (m, 1H, H-3), 0.98 (s, 3H, Me-19), 0.90 (d, 3H, Me-21, $J = 6.0$ Hz), 0.87 (t, 3H, Me-29, $J = 5.6$ Hz), 0.86 (d, 3H, Me-26, $J = 5.6$ Hz), 0.84 (d, 3H, Me-27, $J = 6.6$ Hz), 0.65 (s, 3H, Me-18); **^{13}C-NMR** (67.8 MHz, $CDCl_3$): δ 140.8 (s), 121.7 (d), 71.8 (d), 56.8 (d), 56.0 (d), 50.1 (d), 45.8 (d), 42.3 (t), 42.3 (s), 39.8 (t), 37.2 (t), 36.5 (s), 36.1 (d), 33.9 (t), 31.9 (t), 31.9 (d), 31.7 (t), 29.1 (d), 28.2 (t), 26.1 (t), 24.3 (t), 23.0 (t), 21.1 (t), 19.8 (q), 19.4 (q), 19.0 (q), 18.8 (q), 12.0 (q), 11.9 (q). EI-MS, ^{1}H-NMR and ^{13}C-NMR data agree with the literature[16].

1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone (4): Yellow crystal; **EI-MS (m/e)** 284 [M^+] (9%), 252 (100%), 196 (55%), 168 (45%), 139 (30%); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 13.30 (s, 1H, OH), 9.40 (s, 1H, OH), 8.26-8.30 (m, 2H, H-5 and H-8), 7.77-7.81 (m, 2H, H-6 and H-7), 7.30 (s, 1H, H-4), 4.94 (s, 2H, CH_2), 3.58 (s, 3H, OCH_3); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 187.2 (s) (C = O), 182.5 (s) (C = O), 164.3 (s), 162.1 (s), 134.4 (d, 2C), 134.3 (s), 133.8 (s), 133.7 (s), 127.6 (d), 127.0 (d), 114.4 (s), 110.0 (d), 110.0 (s), 69.2 (t, CH_2-O), 59.6 (q, OCH_3). EI-MS[17] data agree with the literature, ^{1}H-NMR and ^{13}C-NMR agree with the literature[18].

2-Carbomethoxy-3-prenyl-1,4-naphtho-hydroquinone, 1,4-di-O-β-glucoside (5): Colorless needles; **EI-MS (m/e)** 286.1 ([M^+] of aglycone +2) (50%), 254 (100%), 239 (14%), 198 (18%), 165 (6%), 105 (6%), 85 (7%), 73 (17%); **^{1}H-NMR** (400 MHz, CD_3OD): δ 8.55 (bd, 1H, H-5 or H-8, $J = 7.4$ Hz), 8.53 (bd, 1H, H-5 or H-8, $J = 7.7$ Hz), 7.57 (dt, 1H, H-6 or H-7, $J = 7.7$ Hz, $J = 1.1$ Hz), 7.52 (dt, 1H, H-6 or H-7, $J = 7.4$ Hz, $J = 1.1$ Hz), 5.14 (m, 1H, $CH = CMe_2$), 4.84 (m, 9H, overlapped 8xOH and an anomeric proton), 4.65 (d, 1H, anomeric H, $J = 7.7$ Hz), 3.85 (s, 3H, OCH_3), 3.58-3.82 (m, 6H, protons of sugars and $CH_2CH =$), 3.38-3.48 (m, 2H, protons of sugars), 3.26-3.34 (m, 2H, protons of sugars, 3.07-3.13 (m, 2H, protons of sugars), 1.73 (s, 3H, one of C = CMe_2), 1.68 (s, 3H, one of C = CMe_2); **^{13}C-NMR** (100 MHz, CD_3OD): δ 169.2 (C = O), 146.3 (s), 146.0 (s), 131.1 (s), 129.1 (s), 128.7 (s), 126.7 (s), 126.5 (d), 125.8 (s), 125.4 (d), 122.7 (d), 122.5 (d), 122.5 (d), 104.5 (d), 104.3 (d), 76.1 (d), 76.0 (d), 75.9 (d), 75.8 (d), 73.9 (d), 73.6 (d), 70.0 (d), 69.5 (d), 61.3 (t), 60.6 (t), 51.0 (OCH_3), 25.6 (CH_2), 23.9 (CH_3), 16.4 (CH_3) .

EI-MS fragmentation is good agreement with the data given the literature and ^{1}H-NMR is agreement with the data given in the literature[14].

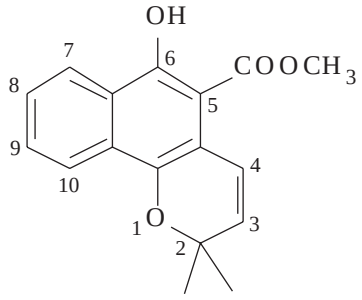
Lucidin-3-O-β-primeveroside (6): Yellow powder; **EI-MS (m/e)** 254 [M^+] (100%), 239 (28%), 207 (14%), 197 (8%), 152 (22%), 129 (28%), 115 (30%); **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 8.25-8.23 (m, 1H, H-5 or H-8), 8.19-8.17 (m, 1H, H-5 or H-8), 7.94-7.92 (m, 2H, H-6 and H-7), 7.47 (s, 1H, H-4), 5.10 (d, 1H, H-1 gluc, $J = 6.6$ Hz), 4.64 (A part of AB system, d, 1H, one of CH_2OH, $J = 11.0$ Hz), 4.56 (B part of AB system, d, 1H, one of CH_2OH, $J = 11.0$ Hz), 4.13 (d, 1H, H-1 xylose, $J = 7.3$ Hz), 3.94 (d, 1H, sugar proton, $J = 9.5$ Hz), 3.72-3.58 (m, 3H, sugar protons), 3.40-3.25 (m, 4H, sugar protons), 3.01 (bt, 1H, sugar proton, $J = 7.0$ Hz), 2.99 (bt, 2H, sugar protons, $J = 10.6$ Hz); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ 187.8 (s) (C = O), 182.2 (s) (C = O), 162.7 (s), 162.6 (s), 135.6 (d), 135.4 (d), 134.5 (s), 133.7 (s), 133.6 (s), 127.7 (d), 127.3 (d), 124.4 (s), 112.1 (s), 107.1 (d), 104.8 (d), 101.5 (d), 77.1 (d), 76.6 (d), 76.4 (d), 74.0 (d, 2C), 70.2 (d), 69.9 (d), 68.7 (t), 66.3 (t), 51.7 (t). ^{1}H-NMR and ^{13}C-NMR agree with the literature[19,20].

1,3-Dihydroxy-2-carboxy-9,10-anthraquinone (Munjistin) (7): Orange substance; **EI-MS (m/e)** 284 [M^+] (0.5%), 240 (44%), 239 (100%), 212 (15%), 184 (18%), 128 (16%), 77 (9%), 69 (12%); ^{1}**H-NMR** (400 MHz, D_2O): δ 7.68 (d, 1H, H-5 or H-8, J = 7.4 Hz), 7.60 (d, 1H, H-5 or H-8, J = 6.7 Hz), 7.49 (t, 1H, H-6 or H-7, J = 7.5 Hz), 7.40 (t, 1H, H-6 or H-7, J = 7.4 Hz), 6.44 (s, 1H, H-4). EI-MS fragmentation is in good agreement with the data given in the literature and ^{1}H-NMR is in agreement with the data given in the literature[15].

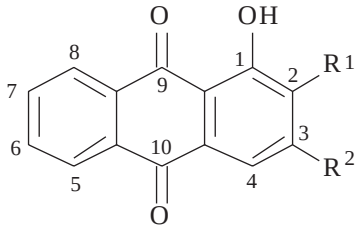
Results and Discussion

The underground parts of *A. taurina* subsp. *caucasica* were extracted with methanol. The extract was fractionated between chloroform and water. The chloroform fraction was subjected to a silica gel column, eluting with n-hexane-ethyl acetate by gradient elution. Similar fractions were collected and combined. As a result of repeated column chromatography and preparative TLC, 4 compounds (**1-4**) were purified. Using Sephadex LH-20, RP-18 and silica gel column chromatography, **5-7** were obtained from the aqueous fraction (Figure).

Characterization of compounds **1-7** was performed by extensive NMR studies plus EI-MS.



1; Mollugin

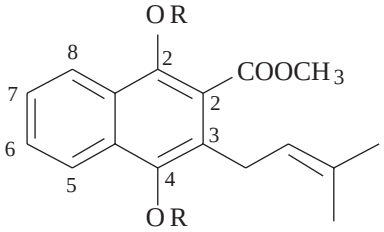


2 ; R^1 = CH_3, R^2 = H

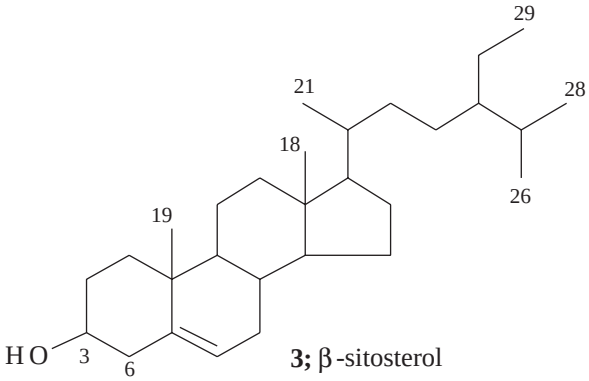
4 ; R^1 = CH_2OCH_3, R^2 = OH

6 ; R^1 = CH_2OH R^2 = O -β-primeveroside

7 ; R^1 = COOH R^2 = OH



5; R = β–D -glucoside



3; β -sitosterol

Figure

The EI-MS spectrum of mollugin **1** showed an M^+ ion peak (284) in regard to its structure. In the

^{1}H-NMR spectrum of mollugin **1**, signals of 2 methyls at C-2 arose at δ 1.48 as 1 singlet, and methoxymethyl at δ 4.01. Olefinic hydrogens were seen as a doublet of doublets at δ 5.68 and 7.12 (J = 9.9 Hz). The signals of 4 protons in the benzene ring were also in accordance with the structure. While H-7 and H-10 resonated as a doublet of doublets, H-8 and H-9 were seen as ddd. All data were in agreement with the data given in the literature[12,14].

As expected, a similarity was seen between the ^{1}H-NMR spectra of the aromatic hydrogens of compounds **4, 6** and **7**. The H-4's of these compounds were shown as singlets. While 4 protons (H-5, H-6, H-7, H-8) of **4** and **6** showed multiplicity in the aromatic area, the same protons of **7** were uncomplicated (H-5 and H-8 as doublets; H-6 and H-7 as triplets). This differentiation probably arises from the diversity of the functional group at C-2 of compound **7**. The signals observed at δ 4.94 and δ 3.58, with 2 and 3 proton intensities, respectively, were assigned to methylene and methyl protons of the methoxymethyl group. Characterization of the sugar moiety in molecule **6** was achieved by comparing with the literature[19]. Eleven carbon signals in the ^{13}C-NMR spectra of **6** belonging to the sugar moiety and chemical shifts and coupling constants measured in ^{1}H-NMR showed that the sugar moiety is primeveroside.

The ^{1}H-NMR spectrum of the aromatic hydrogens of compound **2** differs from those of compounds **4**, **6** and **7**, owing to an AB system made of H-3 and H-4. A methyl singlet of **2** arose at δ 2.39.

$\beta-$ Sitosterol **3** was primarily characterized by comparing its EI-MS spectrum with the data given in the literature. Its ^{1}H and ^{13}C-NMR spectroscopic data were in agreement with the data given in the literature[16].

Two sugar moieties, 1 prenyl group and 1 carboxymethyl group of compound **5** were easily determined from the ^{1}H and ^{13}C-NMR spectra. Signals belonging to 4 protons in the aromatic ring of **5** were also in accordance with the structure. While H-5 and H-8 resonated as broad doublets, H-6 and H-7 were seen as a doublet of triplets in accordance with the structure. An evaluation of the ^{1}H and ^{13}C-NMR spectra of the sugar moiety in compound **5** in comparison with the literature showed that this part should be glucose[15].

In conclusion, in this work we showed the isolation and characterization of 7 compounds from *Asperula taurina* subsp. *caucasica* for the first time.

Acknowledgment

We thank Professor İhsan Çalış for his sending the original NMR spectrum of lucidin-3-O-β-primeveroside for comparison. We especially thank Dr. Hamdullah Kilic for recording the EI-MS spectra of the compounds.

References

1. W.C. Evans, "**Pharmacognosy**", 13th edition, p. 212, English Language Book Society, Balliére Tindall, (1989).
2. A.R. Burnett and R.H. Thomson, **J. Chem. Soc. (C)**, 854-857 (1968).
3. A.R. Burnett and R.H. Thomson, **J. Chem. Soc. (C)**, 2437-2441 (1968).
4. H. Itokawa, Z.Z. Ibraheim, Y.F. Oiao and K. Takeya, **Chem. Pharm. Bull. 41**, 1869-1872 (1993).
5. E.K. Adesogan, **Tetrahedron 29**, 4099-4102 (1973).

6. H. Inouye, Y. Takeda, H. Nishimura, A. Kanomi, T. Okuda and C. Puff, **Phytochemistry 27**, 2591-2598 (1988).
7. M.I. Borisov, **Khim. Prir. Soedin. 10**, 82 (1974), Ref: Chem. Abstr.: **81**: 60805y (1974).
8. H. Itokawa, Y.F. Qiao, K. Takeya and Y. Iitaka, **Chem. Pharm. Bull. 37**, 1670-1672 (1989).
9. M.I. Borisov, V.V. Belikov and T.I. Isakova, **Rastit. Resur., 11**, 351-358 (1975) Ref. Chem. Abstr.: **83**: 190343y (1975).
10. M.I. Borisov, **Rastit. Resur., 11,** 362-368 (1975), Ref. Chem. Abstr.: **83**: 190344z (1975).
11. F. Ehrendorfer and E. Schöbeck-Temesy, **Asperula L.** in "Flora of Turkey and the East Aegean Islands", Vol. 7, pp. 734-767, University Press, Edinburgh (1982). (edited by P.H. Davis).
12. H. Schildknecht, F. Straub and V. Scheidel, **Liebigs Ann. Chem. 7-8**, 1295-1306 (1976).
13. H. Itokawa, K. Mihara, and K. Takeya, **Chem. Pharm. Bull. 31**, 2353-2358 (1983).
14. K. Inoue, Y. Shiobara, H. Nayeshiro, H. Inouye, G. Wilson and M.H. Zenk, **Phytochemistry 23**, 307-311 (1984).
15. H. Itokawa, Y. Qiao and K. Takeya, **Phytochemistry 28**, 3465-3468 (1989).
16. M.D. Greca, P. Monaco and L. Previtera, **J. Nat. Prod. 53**, 1430-1435, (1990).
17. Y. Kawasaki, Y. Goda and K. Yoshihira, **Chem. Pharm. Bull. 40**, 1504-1509 (1992).
18. D.V. Banthorpe and J.J. White, **Phytochemistry 38**, 107-111 (1995).
19. C. Kusamba, E. Federici, Y. De Vicente and C. Galeffi, **Fitoterapia 64**, 18-22 (1993).
20. İ. Calış, D. Taşdemir, C.M. Ireland and O. Sticher, **Chem. Pharm. Bull. 50**, 701-702 (2002).