AkiNik

American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP

American Journal of Essential Oils and Natural Products

ISSN: 2321 9114
AJEONP 2013; 1 (2): 7-10

Received 9-11-2013
Accepted: 13-12-2013

Consolacion Y. Ragasa
Chemistry Department and Center for Natural Sciences and Ecological Research, De La Salle University 2401 Taft Avenue, Manila 1004, Philippines

Giancarlo S. Lorena
Chemistry Department and Center for Natural Sciences and Ecological Research, De La Salle University 2401 Taft Avenue, Manila 1004, Philippines

Emelina H. Mandia
Biology Department and Center for Natural Sciences and Ecological Research, De La Salle University – Manila

Dennis D. Raga
Biology Department and Center for Natural Sciences and Ecological Research, De La Salle University – Manila

Chien-Chang Shen
National Research Institute of Chinese Medicine, 155-1, Li-Nong St., Sec. 2, Taipei 112, Taiwan

Correspondence:
Consolacion Y. Ragasa
Chemistry Department and Center for Natural Sciences and Ecological Research, De La Salle University 2401 Taft Avenue, Manila 1004, Philippines
Email: consolacion.ragasa@dlsu.edu.ph

Chemical constituents of *Abrus precatorius*

Consolacion Y. Ragasa, Giancarlo S. Lorena, Emelina H. Mandia, Dennis D. Raga, Chien-Chang Shen

Abstract

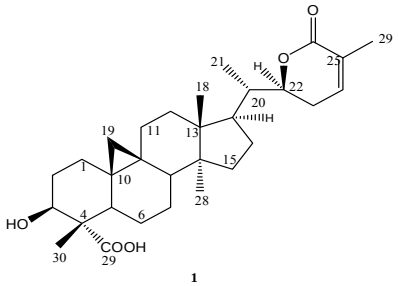
The dichloromethane extract of the pericarp of *Abrus precatorius* afforded abrusogenin, saturated monoglyceride and unsaturated triglyceride. The seeds yielded a mixture of stigmasterol and β-sitosterol in a 4:1 ratio, while the peduncle afforded unsaturated triglyceride. The structures of **1** and saturated monoglyceride were elucidated by extensive 1D and 2D NMR spectroscopy. The structures of triglyceride, stigmasterol and β-sitosterol were identified by comparison of their ^{13}C NMR data with those reported in the literature. Antimicrobial tests on **1**, monoglyceride and triglyceride indicated that they are active against *S. aureus, P. aeruginosa, B. subtilis, C. albicans* and *T. mentagrophytes*. All compounds tested are inactive against *A. niger*, while only **1** is active against *E. coli.*

Keywords: *Abrus precatorius*, Fabaceae, abrusogenin, antimicrobial.

1. Introduction

Abrus precatorius, locally known as saga-saga, rosary pea and crab's eye, is one of the most deadly plants known to man. The seeds contain the protein toxin, abrin which is deadly when ingested even at a small dose. It was reported that as little as 0.00015% of toxin per body can cause fatality in humans[1]. The leaves were found to contain sweet tasting compounds such as abrusoside and glycyrrhizin, which are sweeter than sucrose and have lower caloric value[2]. Abrusoside A-D, which contains abrusogenin as aglycone, exhibited sweetness potencies 30-100 times greater than sucrose[3]. A number of triterpenes were isolated from *A. precatorius,* abrusoside A-E and abrusogenin[4] which are of relevance to our present report. A review on the pharmacognosy, phytochemistry and pharmacology of the leaves of *A. precatorius* has been provided[5].

We report herein the isolation and structure elucidation of abrusogenin (**1**) (Fig 1), saturated monoglyceride and unsaturated triglyceride from the pericarp of *A. precatorius*. The seeds yielded a mixture of stigmasterol and β-sitosterol in a 4:1 ratio, while the peduncle afforded triglyceride.



1

Fig. 1: Chemical structure of abrusogenin (**1**) from *Abrus precatorius* pericarp

2. Materials and Methods

2.1 General

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H-NMR and 150 MHz for ^{13}C-NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh), while the TLC was performed with plastic-backed plates coated with silica gel F_{254}. The plates were visualized with vanillin-H_2SO_4 and warming.

2.2 Plant Material

The fruit of *Abrus precatorius* were collected from Mindoro, Philippines in January 2011. Specimens of the plant were authenticated by one of the authors (E.H.M.) who also collected the sample. A voucher specimen # 804 was deposited at the Biology Department, De La Salle University – Manila.

2.3. Extraction and Isolation

The air-dried pericarp (27.22 gm), seeds (33.35 gm) and peduncle (7.40 gm) of *A. precatorius* were separately ground in an blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrates were concentrated under vacuum to afford crude extracts as follows: pericarp (0.90 gm), seeds (0.87 gm) and peduncle (0.13 gm). The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 (10% increments by volume) as eluents. Fractions were collected and monitored by thin layer chromatography (TLC). Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained.

2.3.1. Isolation of Constituents from the pericarp of *A. precatorius*

The CH_2Cl_2 fraction from the chromatography of the crude CH_2Cl_2 pericarp extract was rechromatographed in petroleum ether. The more polar fractions were rechromatographed in 10% EtOAc in petroleum ether to afford triglyceride (8 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3×) in Et_2O:CH_3CN:CH_2Cl_2 in a 1:1:8 by volume ratio to afford monoglyceride (15 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (5×) in Et_2O:CH_3CN:CH_2Cl_2 in a 1:1:8 by volume ratio to afford **1** (25 mg).

2.3.2. Isolation of Constituents from the seeds of *A. precatorius*

The 20% to 30% acetone in CH_2Cl_2 fractions from the chromatography of the crude seeds extract were combined and rechromatographed in 2.5% EtOAc in petroleum ether, followed by 5% EtOAc in petroleum ether, and then finally with 10% EtOAc in petroleum ether as eluents to afford a mixture of stigmasterol and β-sitosterol (12 mg) in a 4:1 ratio, after washing with petroleum ether. The ratio was based on the integrals in parenthesis of the olefinic protons at δ 5.33 (0.76) for stigmasterol and β-sitosterol and 5.13 (0.59) and δ 5.00 (0.60) for stigmasterol only.

2.3.3. Isolation of Constituents from the peduncle of *A. precatorius*

The 10% acetone in CH_2Cl_2 fraction from the chromatography of the crude peduncle extract was rechromatographed (4×) in 10% EtOAc in petroleum ether to afford triglyceride (25 mg).

2.4. Antimicrobial Assays

The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *Pseudomonas aeruginosa* (UPCC 1244), *Bacillus subtilis* (UPCC 1149), *Escherichia coli* (UPCC 1195), *Staphylococcus aureus* (UPCC 1143), *Candida albicans* (UPCC 2168), *Trichophyton mentagrophytes* (UPCC 4193) and *Aspergillus niger* (UPCC 3701). Compounds **1**, monoglyceride and triglyceride were tested for antimicrobial activity against these microorganisms.

Microbial suspensions were prepared from 24 h old cultures of the bacteria and yeast and from 5-day old culture of the molds. The suspending medium used was 0.1% peptone water. Pre-poured agar plates, about 3 mm thick, were inoculated with the microbial suspension by swabbing the agar surface. Nutrient agar (NA), Glucose Yeast Peptone (GYP) Agar and Potato Dextrose Agar (PDA) were used for bacteria, yeast and molds, respectively. The cotton swab on an applicator stick was dipped into the microbial suspension, rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inocula from the swab. The swab was streaked over the entire agar surface. This procedure was repeated two more times, rotating the plate 60^0 each time to ensure even distribution of the inocula. Three equidistant wells were made on the agar plate using a cork borer (10 mm) and 200 µL of the compound dissolved in 95% ethanol was placed in each hole.

The plates were incubated at room temperature. NA and GYP plates were observed after 24-48 h and PDA plates were observed after 2-5 days. The clearing zone was measured in millimeters and the average diameter of the clearing zones was calculated. The activity index was computed by subtracting the diameter of the well from the diameter of the clearing zone divided by the diameter of the well.

Abrusogenin 1: colorless solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.27, 1.64 (H_2-1), 1.62, 1.82 (H_2-2), 4.10 (H-3, dd, J = 2.4, 8.8 Hz), 1.98 (H-5), 0.96, 1.26 (H_2-6), 1.25 (H_2-7), 1.24 (H-8), 1.12, 2.00 (H_2-11), 1.30 (H_2-12), 1.60, 1.70 (H_2-15), 1.29 (H_2-16), 2.18 (H-17), 0.94 (H_3-18), 0.37 (H-19a, d, J = 1.2 Hz), 0.60 (H-19b, d, J = 1.2 Hz), 0.98 (H_3-21, d, J = 7.2 Hz), 4.77 (H-22, dd, J = 4.8, 11.4 Hz), 1.96, 2.55 (H_2-23), 6.58 (H-24, d, J = 6 Hz), 1.90 (H_3-27, s), 0.85 (H_3-28, s), 1.13 (H_3-30, s); ^{13}C NMR (150 MHz, $CDCl_3$): δ 31.39 (C-1), 29.45 (C-2), 75.35 (C-3), 54.56 (C-4), 44.06 (C-5), 22.96 (C-6), 27.56 (C-7), 47.78 (C-8), 20.02 (C-9), 24.99 (C-10), 26.36 (C-11), 35.39 (C-12), 45.22 (C-13), 48.82 (C-14), 32.58 (C-15), 25.48 (C-16), 47.44 (C-17), 17.84 (C-18), 29.82 (C-19), 40.07 (C-20), 12.82 (C-21), 80.25 (C-22), 27.86 (C-23), 139.71 (C-24), 128.19 (C-25), 166.70 (C-26), 17.14 (C-27), 19.38 (C-28), 181.51 (C-29), 9.05 (C-30).

Monoglyceride: ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.14 dd (6.0, 11.4), 4.19 dd (4.8, 11.4, glyceryl CH_2O), 3.92 (glyceryl CHOH), 3.58, 3.68 (glyceryl CH_2OH), 2.34 t (6.6 α-CH_2), 1.62 (β-CH_2), 1.23-1.25 (CH_2), 0.86 t (7.2, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.12 (CH_3), 22.69, 24.91, 29.12-29.70, 31.92, 34.15, 63.31 (glyceryl CH_2OH), 65.17 (glyceryl CH_2O), 70.27 (glyceryl CHOH), 174.35 (O-C=O).

Triglyceride: ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.28 dd (4.2, 11.4), 4.12 dd (6.0, 11.4, glyceryl CH_2O), 5.32 (glyceryl CHO), 2.31 t (6.6, α-CH_2), 5.33 (olefinic H), 2.75 (double allylic CH_2), 2.03 (allylic, CH_2), 1.25-1.35 (CH_2), 0.87 t (6.6, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 62.08 (glyceryl CH_2), 68.86 (glyceryl CH), 173.29 (C=O α), 172.84 (C=O β), 34.04 (C-2α), 34.18 (C-2β), 24.84 (C-3α), 24.87 (C-3β), 29.08 (C-4α), 29.04 (C-4β), 29.19 (C-5α), 29.26 (C-5β), 29.11 (C-6α), 29.16 (C-6β), 29.62 (C-7α),

29.66 (C-7β), 29.19 (both C-8), 130.01 (C-9α), 129.9 (C-9β), 128.06 (C-10α), 128.07 (C-10β), 25.62 (both C-11), 127.88 (C-12α), 127.74 (C-12β), 130.21 (both C-13), 27.19 (both C-14), 29.36 (both C-15), 31.52 (both C-16), 22.57 (both C-17), 14.07 (both C-18).

Stigmasterol: ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.50 (m, H-3), 5.33 (d, 4.8, H-5), 0.68 (s, CH_3-18), 0.99 (s, CH_3-19), 1.01 (d, 6.6, CH_3-21), 5.13 dd (8.4, 15.6, H-22), 5.00 dd (8.4, 15.0, H-23), 0.84 d (6.6, CH_3-26), 0.83 d (6.0, CH_3-27), 0.80 t (6.0, CH_3-29); ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.80 (C-3), 42.29 (C-4), 140.75 (C-5), 121.72 (C-6), 31.89 (C-7), 31.89 (C-8), 50.14 (C-9), 36.50 (C-10), 21.07 (C-11), 39.66 (C-12), 42.19 (C-13), 56.75 (C-14), 24.35 (C-15), 28.91 (C-16), 55.93 (C-17), 12.04 (C-18), 19.38 (C-19), 40.48 (C-20), 21.07 (C-21), 138.31 (C-22), 129.26 (C-23), 51.23 (C-24), 31.90 (C-25), 21.20 (C-26), 18.96 (C-27), 25.40 (C-28), 12.24 (C-29).

β-Sitosterol: ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.50 (m, H-3), 5.35 (d, 4.8, H-5), 0.66 (s, CH_3-18), 0.99 (s, CH_3-19), 0.93 (d, 6.6, CH_3-21), 0.84 d (6.6, CH_3-26), 0.83 d (6.0, CH_3-27), 0.86 t (6.0, CH_3-29); ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.80 (C-3), 42.29 (C-4), 140.75 (C-5), 121.72 (C-6), 31.89 (C-7), 31.89 (C-8), 50.14 (C-9), 36.50 (C-10), 21.07 (C-11), 39.76 (C-12), 42.19 (C-13), 56.75 (C-14), 24.35 (C-15), 28.24 (C-16), 56.04 (C-17), 11.97 (C-18), 19.38 (C-19), 36.14 (C-20), 18.76 (C-21), 33.93 (C-22), 26.04 (C-23), 45.84 (C-24), 29.13 (C-25), 19.02 (C-26), 19.81 (C-27), 23.05 (C-28), 11.85 (C-29).

3. Results and Discussion

3.1. Structure Elucidation

Silica gel chromatography of the dichloromethane extract of the pericarp of *Abrus precatorius* afforded abrusogenin (**1**), saturated monoglyceride and triglyceride. The seeds yielded a mixture of stigmasterol and β-sitosterol in a 4:1 ratio, while the peduncle afforded triglyceride. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy as follows.

The ^{1}H NMR spectrum of **1** (see experimental part) gave resonances for a cyclopropyl methylene doublets at δ 0.60 (J = 1.2 Hz) and 0.37 (J = 1.2 Hz); an allylic methyl at δ 1.90 (br s); a methyl doublet at δ 0.98 (J = 7.2 Hz); three methyl singlets at δ 0.85, 1.90, 1.13; an olefinic proton at δ 6.58 (d, J = 6.0 Hz); and oxymethine protons at δ 4.77 (dd, J = 4.8, 11.4 Hz) and δ 4.10 (dd, J = 2.4, 8.8 Hz). The ^{13}C NMR spectrum of **1** (see experimental part) gave resonances for thirty carbons with the following functionalities: oxymethine carbons at δ 75.35 and 80.25; olefinic carbons at δ 128.19 and 139.71; a carbonyl carbon of a conjugated lactone at δ 166.70; a carboxylic acid at δ 181.51; ten methylene, five methine, five methyl and four quaternary carbons. These resonances are characteristics of a cycloartenol derivative with an olefin, an alcohol, a carboxylic acid and a lactone functionalities.

The COSY spectrum of **1** indicated five isolated spin systems as follows: H_2-1/H_2-2/H-3; H-5/H_2-6/H_2-7/H-8; H_2-11/H_2-12; H_2-15/H_2-16/H-17/H-20/H_3-21,H-22/H_2-23/H-24; H_2-19 (Fig. 1). Protons attached to carbons were assigned (see experimental part) from HMQC 2D NMR data and the structure of **1** was elucidated by analysis of the HMBC 2D NMR data: key HMBC correlations are shown in Fig. 2.

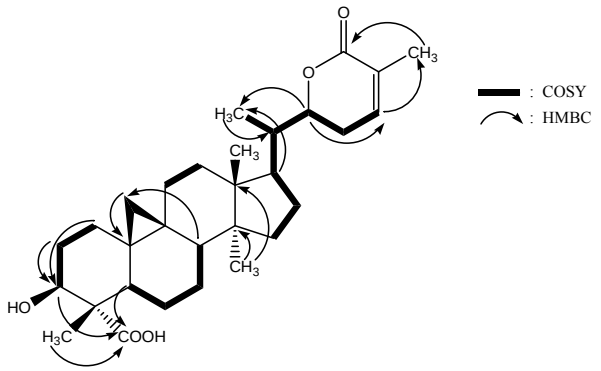


Fig 2: ^{1}H-^{1}H COSY and ^{1}H-^{13}C long-range correlation of **1**

Thus, the oxymethine carbon was assigned to C-3 on the basis of long-range correlations between the methyl singlet at H_3-30, H_2-1, H_2-2, H-5 and this carbon. The cyclopropyl methylene protons were attached to C-19 since these protons showed correlations with C-9, C-10, C-1, C-5, C-8 and C-11. The double bond was assigned to C-24 due to long-range correlations between H_3-27, H-22 and H_2-23 and this carbon. The carbonyl carbon of the lactone was attributed to C-26 based on long-range correlations between this carbon and H_3-27, H-24, H-22. The carboxylic acid was assigned to C-29 due to correlations observed between H-3, H-5, H_3-30 and this carbon. All long-range correlations are consistent with the structure of **1**.

The relative stereochemistry of **1** was deduced from the NOESY spectrum as follows. The oxymethine proton (H-3) was close in space to the methine proton (H-5), which correlated with the methyl singlet (H_3-28). The methine proton (H-17) was close to the methyl doublet (H_3-21), which in turn correlated with the oxymethine proton (H-22). This indicated that H-3, H-5, H_3-28, H-17, H-22 and H_3-21 are on the same face of **1**. On the opposite face of the molecule, the methyl singlet (H_3-30) was close in space to one of the cyclopropyl methylene protons (H-19a). The other cyclopropyl methylene proton (H-19b) was close to the methine proton (H-8), which in turn correlated with the methyl singlet (H_3-18), which was finally correlated with another methine proton (H-20). Thus, **1** has the relative configuration shown in Fig. 3.

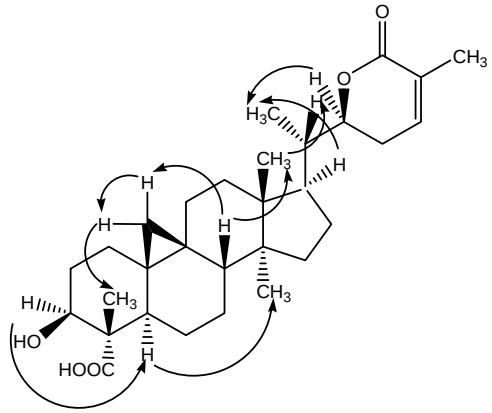


Fig 3: NOESY Correlations of **1**

Literature search revealed that **1** is abrusogenin as confirmed by similar ^{1}H NMR and ^{13}C NMR data[3]. However, only the methyl, oxymethine and cyclopropyl ^{1}H NMR resonances were assigned in the literature.

The structure of monoglyceride was elucidated by extensive 1D and 2D NMR spectroscopy and gave similar ^{1}H NMR and ^{13}C NMR resonances (see experimental) to glycerol monolaurate[6]. Triglyceride gave similar ^{13}C NMR resonances (see experimental part) to trilinolein[7]. The structures of stigmasterol and β-sitosterol were confirmed by similar ^{1}H NMR and ^{13}C NMR data (see experimental part) with those reported in literature[8].

3.2. Antimicrobial Assay

As part of our continuing search for antimicrobial compounds from Philippine medicinal plants, **1**, monoglyceride and triglyceride were tested for possible antimicrobial activities by the agar well method. Results of the study (Table 1)

Table 1. Antimicrobial Activity of Compounds (**1**-**3**).

Microorganism	Compound[a] (30 µg)	Clearing Zone (mm)[b]	Activity Index (AI)
E. coli	**1**	-[c]	
	monoglyceride	-[c]	
	triglyceride	12	0.2
	Chloramphenicol[d]	30	4.0
P. aeruginosa	**1**	11	0.1
	monoglyceride	11	0.1
	triglyceride	12	0.2
	Chloramphenicol[d]	15	1.5
S. aureus	**1**	18	0.8
	monoglyceride	12	0.3
	triglyceride	13	0.3
	Chloramphenicol[d]	33	4.5
B. subtilis	**1**	13	0.3
	monoglyceride	12	0.2
	triglyceride	12	0.2
	Chloramphenicol[d]	20	2.3
C.albicans	**1**	13	0.3
	monoglyceride	13	0.3
	triglyceride	12	0.2
	Canesten[e], 0.2 g	18	0.8
T. mentagrophytes	**1**	13	0.3
	monoglyceride	11	0.1
	triglyceride	12	0.2
	Canesten[e], 0.2 g	55	4.5
A. niger	**1**	-[c]	
	monoglyceride	-[c]	
	triglyceride	-[c]	
	Canesten[e], 0.2 g	23	1.3

[a]Sample – 10 mm well diameter. [b]Average of three replicates. [c]No clearing zone. [d]Chloramphenicol disk - 6-mm disc. [e]Contains 1% clotrimazole.

indicated that except for *E. coli* and *A. niger,* **1** and monoglyceride were active against all the microorganisms tested, while triglyceride was inactive only against *A. niger*. Triterpene **1** is the most active against *S. aureus* and *T. mentagrophytes* with activity index (AI) of 0.8 and 0.3, respectively. Triglyceride is more active (AI = 0.2, 0.2 and 0.2) than monoglyceride (AI = 0, 0.1 and 0.1) against *E. coli, P. aeruginosa* and *T. mentagrophytes,* respectively. On the other hand, monoglyceride is more active (AI = 0.3) than triglyceride (AI = 0.2) against *C. albicans.*

4. References

1. MacDonald G, Ferrell J, Sellers B, Langeland K, Duperron-Bond T, Ketterer-Guest T. Invasive Species Management Plans for Florida. University of Florida, IFAS Extension, Circular, 2008, 1529.
2. Choi Y-H, Kinghorn AD, Shi X, Zhang H, Teo B. Abrusoside A: new type of highly sweet triterpene glycoside. Journal of Chemical Society, Chemical Communications 1989; 887-888.
3. Choi Y-H, Hussain R, Pezzuto J, Kinghorn AD, Orton JF. Abrusosides A-D, four novel sweet-tasting triterpene glycosides from the leaves of *Abrus precatorius*. Journal of Natural Products 1989; 52(5):1118-1127.
4. Kennelly E, Cai L, Kim N-C, Kingborn AD. Abrusoside E, a further sweet tasting cycloartane glycoside from the leaves of *Abrus precatorius.* Phytochemistry 1996; 41(5):1381-1383.
5. Solanki A, Zave M. Pharmacognosy, phytochemistry and pharmacology of *Abrus precatorius* leaf: A review. International Journal of Pharmaceutical Sciences Review and Research 2012; 13:71-76.
6. Gandhi M, Mostashari A, Karegar M. Efficient synthesis of α-monoglycerides via solventless condensation of fatty acids with glycerol carbonate. Journal of American Oil Chemists' Society 2007; 84:681-685.
7. Alemany LB. Using simple ^{13}C NMR linewidth and relaxation measurements to make detailed chemical shift assignments in triacylglycerols and related compounds. Chemistry of Physics and Lipids 2002; 120(1-2):33-44.
8. Cayme JMC, Ragasa CY. Structure elucidation of β-stigmasterol and β-sitosterol from *Sesbania grandiflora Linn. Pers.* and β-Carotene from *Heliotropium indicum* Linn. by NMR spectroscopy. Kimika 2004; 20(1/2):5-12.