Table: 1st order kinetic parameters of urea/urease reaction in a multiple W/O/W emulsion system as a function of the nature of lipophilic phase (n = 3)

Lipophilic phase in W/O/W emulsion	Kinetic parameters (1st order)		
	k (min^{-1})	Correlation coefficient	$t_{1/2}$ (min)
Liquid paraffin	5×10^{-4}	0.984	1386
Isopropylmiristate	1.1×10^{-3}	0.984	630
Olive oil	5×10^{-4}	0.982	1386
Castor oil	6×10^{-4}	0.984	1155
Almond oil	3×10^{-4}	0.989	2310
Aqueous solution	6.4×10^{-3}	0.986	108

enzyme intact and acts as a rate-controlling factor of the mass transport toward the internal aqueous phase. Further investigations involving study of the effect of membrane surface and thickness on the effective kinetic coefficient are in process.

In conclusion, the emulsion system itself affects the kinetic parameters of an enzymatic process. Modification of the composition of the middle oil layer in a multiple W/O/W system, used as a medium for an enzymatic process, could be considered as an approach for controlling the rate of the enzymatic process.

References

1 Li., N. N.: US Patent 3,410,794 (1968).
2 May, S. W.; Li N. N.: Enzyme Eng. **2**,77 (1974)
3 Matsumoto, S.; Won Kang, W.: Agric. Biol. Chem. **52**, 2689 (1988)
4 Kato, K.; Yamasaki, N.; Il. N.: J. Chem. Eng. Jpn. **24**, 709 (1991)
5 Bornscheuer, U.; Padmanabhan, P.; Scheper, T. in: Arshady, R. (Ed.): Microspheres, Microcapsules & Liposomes, Vol. **1**, p. 543, Citus Books, London 1999
6 Cahn, R. P.; Ho, W. S.; Li, N. N. in Grossiord, J. L. ; Seiller, M (Eds.): Multiple emulsions, Structure, Properties and Applications, p. 373, Editions de Sante, Paris 1998
7 Scheper, T.: Adv. Drug Dev. Rev. **4**, 209 (1990)
8 Fredro-Kumbaradzi E.: Ph. D. Thesis, University St. Kiril&Metodij, Skopje (1996)
9 Matsumoto, S.; Kita,Y.; Yonezawa,D.: J. Colloid Interface Sci. **57**, 353 (1976)

Department of Pharmacognosy[1], Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt, Department of Chemistry[2], Faculty of Science, Beirut Arab University, Beirut, Lebanon

Three new butyl glycosides from *Inula crithmoides* L. growing in Egypt

A. M. El-Lakany[1], M. A. Aboul-Ela[1], H. M. Hammoda[1], M. M. Abdul-Ghani[2]

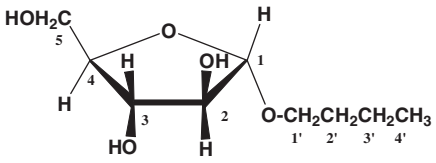
Received April 29, 2003, accepted June 5, 2003

Dr. Abdalla M. El-Lakany, Department of Pharmacognosy, Faculty of Pharmacy, Khartoum Square, Alexandria University, Alexandria, Egypt

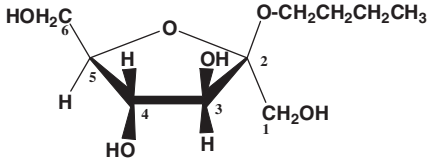
Pharmazie 58: 940–942 (2003)

Three new glycosides have been isolated from *Inula crithmoides* L. Structure elucidation of the isolated compounds was established by the application of spectroscopic analyses including, 1 D and 2 D NMR spectroscopy and MS.

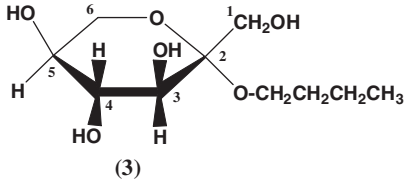
The genus *Inula* (Asteraceae) comprises 200 species [1]. The major secondary metabolites of the genus are sesquiterpene lactones mainly eudesmanolides [2], monoterpenes [3], diterpenes [4] and flavonoids of diverse chemical structures [5]. Several pharmacological activities are attributed to these secondary metabolites, including treatment of asthma, dysentery and inflammatory diseases [6, 7]. Inulin, a fructose polymer and fructo-oligosaccharides are of common occurrence in Asteraceae [8, 9]. Fructo-oligosaccharides have been shown to exhibit beneficial effects by stimulating the growth of Bifidobacteria in the human colon, by suppression of putrefactive pathogens, and by reduction of serum cholesterol concentration [10]. In addition, fructose is used as a food by diabetic patients and



(1)



(2)



(3)

Isolated Compounds

also is recommended in infant feeding formulas. In Egypt, the genus *Inula* is represented by two species [11]: *I. viscosa* and *I. crithmoides*. We previously reported the isolation of two new methoxylated flavonols from *I. crithmoides* L. [12], in addition to 1,5-di-*o*-caffeoylquinic acid [13].

In continuation of our investigation of the Egyptian *I. crithmoides*, we report here the isolation and structure elucidation of three uncommon sugar derivatives (**1**–**3**) obtained for the first time from a natural source. Their chemical structures were identified as 1-butyl ether-β-D-xylofuranoside (**1**), 2-butyl ether-α-D-fructofuranoside (**2**) and 2-butyl ether-β-D-fructopyranoside (**3**).

Different chemical and spectral data as well as enzymatic hydrolysis indicated the glycosidic nature of the isolated compounds. The β-linkage of compound **1** was indicated by enzymatic hydrolysis using emulsin enzyme. The molecular formula of **1** was determined to be $C_9H_{18}O_5$. The MS revealed the presence of the molecular ion peak at m/z 206, while the ^{13}C NMR spectrum revealed the presence of nine carbon signals. DEPT-135° experiment showed these carbons to be 1 CH_3, 4 CH_2 and 4 CH. The ^{1}H NMR spectrum of compound **1** showed a characteristic signal for the β-anomeric proton at δ 4.68 (d, J = 5.1 Hz), with its corresponding carbon signal resonating at δ 105.66 as observed from the HMQC spectrum. HMBC experiment indicated a strong relation for the anomeric proton with the two carbons resonating at δ 80.02 and 74.90 assigned for C-2 and C-3, respectively. The non-sugar part was confirmed to be a butyl group through the appearance of four resolved sets of protons at δ 0.83 (t, J = 7.3 Hz, 3 H), 1.27 (m, 2 H), 1.44 (m, 2 H) and 3.52 (m-2 H) along with their corresponding carbon signals observed at δ 11.65, 16.69, 29.11 and 59.20 respectively, as shown by correlation of 1 D and 2 D-NMR experiments. The MS revealed the presence of a mass fragment at m/z 149 due to loss of the butyl group. Accordingly, the sugar should be a pentose derivative. Comparing the ^{13}C NMR data of **1** with those reported for sugars [14] it was found that they were almost identical to those reported for β-D-xylofuranoside, confirming compound **1** to be 1-butyl ether-β-D-xylofuranoside.

1H NMR spectra of **2** and **3** were free from anomeric proton signals, indicating their ketonic nature. MS and NMR data indicated the molecular formula of both compounds to be $C_{10}H_{20}O_6$. DEPT-135 experiments indicated the ten carbons to be: 1 CH_3, 5 CH_2, 3 CH and one quaternary. Similar to **1**, spectral data of **2** and **3** indicated the presence of a butyl moiety as the aglycone part. ^{1}H NMR spectrum of **2** in DMSO-d_6, before and after addition of D_2O, indicated the presence of two CH_2–OH and two CH–OH groups. This suggested the sugar to be in the furanose form. Comparing ^{13}C NMR data of **2** with those reported for sugars [14], indicated the presence of an α-D-frutcofuranosyl moiety. Enzymatic hydrolysis by invertase enzyme confirmed the α-linkage in **2**, accordingly, **2** is 2-butyl ether-α-D-fructofuranoside.

Enzymatic hydrolysis of **3** by emulsin enzyme indicated its β-configuration, while the low value of chemical shifts in the ^{13}C NMR spectrum pointed to its existance in the pyranose form [14]. Comprarison between the data of the sugar part of **3** with those reported for β-D-fructopyranose, indicated their structural similarity. Thus, **3** is 2-butyl ether-β-D-fructopyranoside. Finally the complete assignment of protons and carbons was done using different 2 D-NMR techniques.

Experimental

1. General procedures

NMR spectra were run in DMSO-d_6 and $CDOD_3$, using a Brucker Avance 300 MHz spectrometer. EIMS spectra were recorded on GC coupled with a Shimadzu 8080A mass spectrometer. Silica gel (70–230 mesh) for CC and silica gel plates (precoated 60 F-254 Merck). The spots were located using anisaldehyde/H_2SO_4 spray reagent followed by heating at 105 °C for 5 min.

2. Plant material

The flowering plant of *I. crithmoides* L. was collected in July 2001, from Rosette and identified by Prof. Dr. Nabil El-Hadidy, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Egypt.

3. Extraction, isolation and purification of compounds 1–3

The air dried powdered aerial parts (3 kg) were macerated in EtOH at room temperature until exhausion. The combined alcoholic extracts were concentrated under reduced pressure to about 500 ml, then diluted with 200 ml H_2O. The hydro-alcoholic extract was filtered and the filterate was subjected to succesive fractionation using light petroleum, $CHCl_3$ and ethyl acetate. The aqueous solution was concentrated and extracted with MeOH, then filtered. The residue obtained after removal of MeOH (3 g) was subjected to CC on silica gel eluted with $CHCl_3$/MeOH gradient elution systems. Fractions (11–13) obtained using 15% MeOH in $CHCl_3$ were subjected to pTLC fluorescent silica gel plates using $CHCl_3$-MeOH (9 : 1) as developing system. The first zone with $R_f = 0.6$ was scrapped off and eluted with $CHCl_3$–MeOH (1 : 2) to give 3 mg of compound **1**, while the second zone ($R_f = 0.52$) afforded 10 mg of compound **2**. Fraction 14 (20% MeOH in $CHCl_3$) was purified using pTLC silica gel plates using $CHCl_3$-MeOH (8.5 : 1.5) as a developing system, the zone with R_f value = 0.46 was scrapped off to give 15 mg of compound **3**.

3.1. 1-Butyl ether-β-D-xylofuranoside (1)

EIMS m/z (rel. int.) 206 (3.9), 149 (16.7), 133 (7.0), 110 (8.1), 103 (9.8), 57 (100). ^{1}H NMR (DMSO-d_6) δ 4.68 (1 H, d, J = 5.1 Hz, H-1), 3.52 (2 H, m, CH_2-1′), 3.86 (1 H, m, H-4), 3.75 (1 H, m, H-3), 3.69 (1 H, m, H-2), 3.62 (1 H, m, H-5), 3.31 (1 H, m, H-5), 1.44 (2 H, m, CH_2-2′), 1.27 (2 H, m, CH_2-3′), 0.83 (3 H, t, J = 7.3 Hz, CH_3-4′). ^{13}C NMR (DMSO-d_6), δ 105.66 (C-1), 80.02 (C-2), 74.90 (C-3), 81.43 (C-4), 64.36 (C-5), 59.20 (C-1′), 29.11 (C-2′), 16.69 (C-3′), 11.65 (C-4′).

3.2. 2-Butyl ether-α-D-fructofuranoside (2)

EIMS m/z (rel. int.) 236 (2.1), 205 (11.3), 180 (3.0), 163 (4.0), 149 (14.4), 145 (4.5), 133 (6.9), 103 (12.1), 85(11.8), 73 (30.9), 56 (100). ^{1}H NMR (DMSO-d_6) δ 3.84 (1 H, d, J = 4.8 Hz, H-3), 3.46 (1 H, d, J = 11.8 Hz, H-1), 3.37 (1 H, m, H-1), 3.67 (1 H, m, H-4), 3.63 (1 H, m, H-5), 3.53 (1 H, dd, J = 12.3, 2.6 Hz, H-6), 3.41 (1 H, m, H-6), 3.38 (2H, m, CH_2-1′), 1.44 (2 H, m, CH_2-2′), 1.32 (2 H, m, CH_2-3′), 0.86 (3 H, t, J = 7.2 Hz, CH_3-4′). ^{13}C NMR (DMSO-d_6) δ 59.18* (C-1), 106.47 (C-2), 80.60 (C-3), 76.02 (C-4), 81.56 (C-5), 59.49* (C-6), 60.51 (C-1′), 31.14 (C-2′), 18.12 (C-3′), 13.23 (C-4′).

* Exchangable values.

3.3. 2-Butyl ether-β-D-fructofuranoside (3)

EIMS m/z (rel. int.) 236 (1.8), 205 (24.7), 179 (9.0), 163 (5.3), 145 (6.5), 133 (7.8), 112 (10.7), 103 (17.2), 85 (32.4), 73 (81.3), 57 (100). ^{1}H NMR (CD_3OD) δ 3.73 (2 H, m, CH_2-1), 3.93 (1 H, d, J = 9.7 Hz, H-3), 3.79 (1 H, dd, J = 9.7, 3.4 Hz, H-4), 3.85 (1 H, m, H-5), 3.66 (1 H, m, H-6), 3.76 (1 H, m, H-6), 3.52 (2 H, m, CH_2-1′), 1.55 (2 H, m, CH_2-2′), 1.41 (2 H, m, CH_2-3′), 0.95 (3 H, t, J = 7.3 Hz, CH_3-4′). ^{13}C NMR (CD_3OD), δ 61.37 (C-1), 99.60 (C-2), 68.53 (C-3), 69.15 (C-4), 69.00 (C-5), 63.12 (C-6), 59.61 (C-1′), 31.27 (C-2′), 18.37 (C-3′), 12.19 (C-4′).

References

1 Harbone, J. B.; Turner, B. L.: Plant Chemosystematics, Academic Press, London 1984
2 Mahmoud, Z. F.; Abdel-Salam, N. A.; Sarg, T. M.; Bohlmann, F.: Phytochemistry **20**, 735 (1981)
3 Gonzalez, A. G.; Barrera, J. B. Rosas, F. E.; Hernandez, A. C. Y.; Espineira, J.; Joseph-Nathan, P.: Phytochemistry **25**, 2889 (1986)
4 Shao, Y.; Bai, N. S.; Zhou, B. N.: Chin. Chem. Lett. **5**, 757 (1994) Through C. A. 122, 81778x (1995)
5 Wollenweber, E.; Mayer, K.; Roitman, J. N.: Phytochemistry **30**, 2445 (1991)
6 Ahmad, V. U.; Ismail, N.: Phytochemistry **30**, 1040 (1991)
7 Zhou, B. N.; Bai, N. S.; Lin, Z. L.; Cordell, G. A.: Phytochemistry **36**, 721 (1994)

8 Heywood, V.; Harbone, J. B.; Turner, B. L.: The Biology and Chemistry of The Compositae, 1 and 2 Academic Press, London, New York, San Francisco 1977
9 Campbell, J. M.; Bauer, L. L.; Fahey, G. C., Hogarth, A. J. C. L.; Walf. B. W.; Hunter, D. E.: J. Agric. Food. Chem. **45**, 3076 (1997)
10 Gibson, G. R.; Roberfroid, M. B.: Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics: J. Nutr. **125**, 1401 (1995)
11 Tackholm, V.: Students Flora of Egypt, Anglo-Egyptian Book-Shop Cairo 1974
12 EL-Lakany, A. M.; Abou Ela, M. A.; Hammoda, H. M.; Ghazy, N. M. and Mahmoud, Z. F.: Pharmazie **51**, 435 (1996)
13 Hammoda, H. M.: MS Thesis; Faculty of Pharmacy, Alexandria University 1996
14 Kalinowsti, H. O.; Burger, S.; Braun, S.; ^{13}C Spectroskopie; George Thieme Verlag. Stuttgart, New York 1984