ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 2, 3 SECTIO DDD 2011

[1]Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Faculty of Pharmacy, Medical University of Lublin
[2]Main Pharmaceutical Inspectorate, Warsaw

DARIUSZ MATOSIUK[1], DANIEL GRALAK[2], MARTA RYZNAR[1]

Lipophilicity of natural coumarines

Lipofilność naturalnych kumaryn

Natural and synthetic coumarins are well known for their broad presence in the nature and biological activity [2]. They are derivatives of benzo[α]pyrone (2H-chromen-2-one) and chemically are lactones of o-hydroxycynnamic acid [7]. The simplest one, which also gave the name to the whole class is coumarin, isolated first by Vogel in 1920 [4]. They can be divided into four classes and additional four subclasses [9,10] – simple coumarins (coumarin, umbelipheron, herniarin, scopoletin, esculetin, fraxetin, ostol, ostenol, dicoumarol, etc.); isocoumarins (feralolide, mellein, polygonolide, paepalantin, sescandelin, etc.); furanocoumarins derived into two subclasses of psolaren ((2H-furan[3,2-g]benzo[b]pyran-2-on) - psolaren, bergapten, xantotoxin, imperatorin, isopimpinelin, marmesin, etc.) and angelicin ((2H-furan[2,3-h]benzo[b]-pyran-2-on) - angelicin, pimpinelin, isobergapten, etc.), and pyranocoumarins also divided into two subclasses of xantiletin ((2H-pirano[3,2-g]benzo[b]pyran-2-on) – xantiletin, calanolide, etc.) and sesselin ((2H-pirano[3,2-h] benzo[b]pyran-2-on) – sesselin, laserpitin, visnadin, samidin, etc.) (Fig. 1).

Biological activity of natural coumarins is very broad, from antimicrobial to anticancer. In the same time coumarins exhibit also severe side effects, considering especially high toxicity of isocoumarins [1] and phototoxicity of furanocoumarins [13,5].

Between main classes of coumarins great structural differences like lack or presence of additional heterocyclic rings occur, but in general within the class coumarins differ mainly by location of the hydroxylic groups and their character – free hydroxylic or alkoxylic. Therefore it is sometimes hard to explain activity profile as well as behavior in the chromatographic process. Separation of the coumarins on normal phases is not easy and allows rather separation of polar and non-polar fractions than particular specimens. That behavior can be explained on the basis of effects location of the oxygen atom (in hydroxylic or alkoxylic groups) can have on the polar properties of particular molecule and would affect much more the lipophilicity than other properties important for retention.

Base on that observation we planned to investigate the lipophylic properties of some coumarins by measuring and calculating the partition coefficient by RP TLC technique in different solvent systems with measurements standardization.

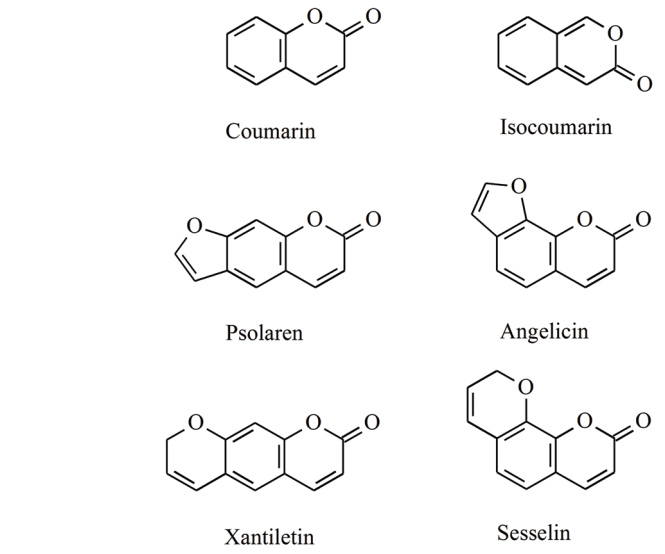

Fig. 1. Leading structures for four main classes and subclasses of coumarins

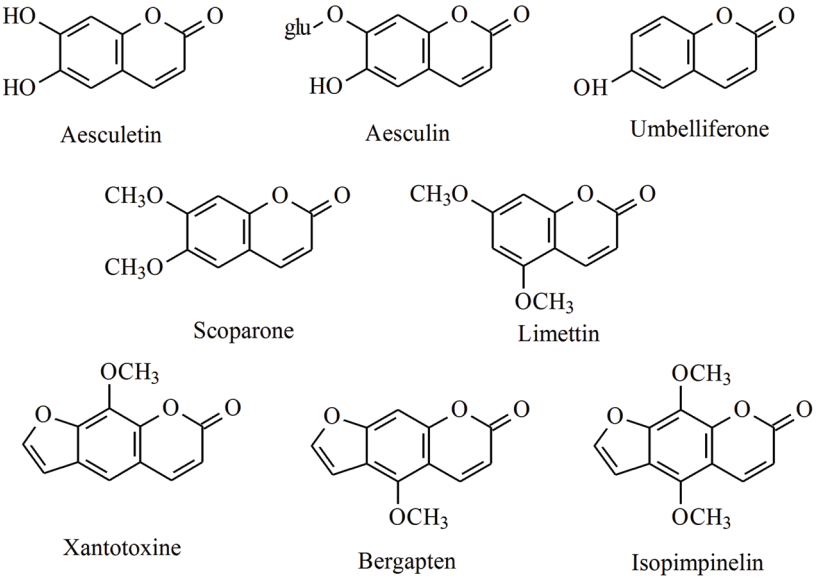

Fig. 2. Structures of coumarins and furanocoumarins investigated

L i p o p h i l i c i t y d e t e r m i n a t i o n. Lipophilicity is one of the molecule properties which determine many aspects of their pharmacology and pharmacokinetics [12]. Nowadays lipophilicity is determined by chromatographic methods – TLC or HPLC in reversed-phase techniques [3]. In comparison to the classical water-n-octanol extraction method they are much faster, reliable and repeatable. The reversed phase, especially C-8 and C-18 imitate the lipid bi-layer making cell wall. Interaction with that phase correspond therefore with the ability to pass the cell wall and activate or inhibit respective molecular target. Since now recognition of the lipophilicity also as one of very important features responsible for selective ligand-molecular target interaction was confirmed [8].

In our research we used C-18 RP-TLC method with water-polar modifier (acetone, methanol, 1,4-dioxane) solvent systems. Measuring the retention factors in different polar modifier concentrations followed by calculation of the chromatographic lipophilicity indices RM and their decimal logarithms. According to Soczewiński-Wachtmeister equation [14] correlation of the logarithm of RM with polar modifier concentration should be linear in the certain concentration range (usually between 50-80%) and allow calculation of RMw values for 0% concentration of polar modifier.

The measurements were standardized by the use of six standards of known lipophilicity [6] and respective calibration/standardization equations [11].

MATERIALS AND METHODS

Lipophilicity was measured for eight coumarins and furanocoumarins – Aesculetin (E1), Aesculin (E2), Umbelliferone (U), Scoparone (S), Limettin (L), Xantotoxine (fc) (X), Bergapten (fc) (B), Isopimpinelin (fc) (I) (Fig. 2).

Samples were obtained from Department of Pharmacognosy, Medical University of Lublin. The 1mg/ml solution of coumarins was applied on the 10 x 10 cm SiO2 C18 RP-TLC plates (Merck, Darmstadt, Germany) and developed on the 8 cm distance in horizontal chamber (sandwich type) (Chromdes, Lublin, Poland). Eluents contained water and polar modifiers – aceton (A), methanol (M) or dioxane (D) in the 55-80 % range with 5 % increments. Chromatograms were visualized in 254 nm U V light and the retention factors measured. They were further recalculated into R_M lipophilicity indices with eq. 1.

$$R_M=\log (1-Rf/Rf) \text{ (eq. 1)}$$

Correlation of RM indices with concentration of the polar modifier exhibited linear character in measured range of concentrations, according to the Soczewinski-Wachtmeister equation [12] (eq. 2)

$$R_M = R_{Mw} – S\varphi \text{ (eq. 2)}$$

where, S – slope of the regression curve; φ – concentration of the polar modifier;

R_{Mw} values in this equation represent the value of lipophilicity indices extrapolated to pure water – the TLC lipophilicity. It was then recalculated into experimental lipophilicity by standardization of the methodological. Standardization is a calibration of the method technique

in which standards of known lipophilicity are used. By those individual effects of measurement conditions, both from the solvent system used and stationary phase-analyte interactions can be made universal. As standards isatin (S1), 2,4-dichloroacetanilide (S2), 2,6-dichloroacetanilide (S3), 3,4-dichloroaniline (S4), 2,6-dichloroaniline (S5) and biphenyl (S6). Choice of standards allowed covering both as wide as possible range of lipophilicity (logP = 0.83-4.01) but also the broadest spectrum of possible molecular interactions (basic and acidic nitrogen atoms, donors and acceptors of hydrogen bonds, polar and dispersive interactions). Their lipophilicity was measured under exactly the same conditions as coumarins analyzed (Table 1). For coumarins investigated and standards all measurements were triplicated and mean values were used further. R_{Mw} indices for standards were correlated with their literature lipophilicity [6], producing calibration/ standardization equations for each solvent system used (eq. 3-5).

$$\log P_{EXP\_A} = 1{,}5481\ R_{Mw} - 1{,}2394 \qquad r^2 = 0{,}9763 \quad \text{(eq. 3)}$$

$$\log P_{EXP\_D} = 1{,}0801\ R_{Mw} + 0{,}1769 \qquad r^2 = 0{,}9948 \quad \text{(eq. 4)}$$

$$\log P_{EXP\_M} = 1{,}1047\ R_{Mw} - 0{,}445 \qquad r^2 = 0{,}9702 \quad \text{(eq. 5)}$$

Table 1. Values of parameters for standards used

		r2	RMw	logP	S
S1	A	0.9773	1.38	0.83	2.62
	D	0.9568	0.65	0.83	1.66
	M	0.9557	1.14	0.83	1.91
S2	A	0.9773	1.54	1.32	2.81
	D	0.974	1.87	2.18	2.58
	M	0.9683	2.73	2.18	3.32
S3	A	0.99	2.20	2.18	3.19
	D	0.9921	0.96	1.32	2.00
	M	0.9538	1.51	1.32	2.29
S4	A	0.9909	2.70	2.69	3.75
	D	0.9956	2.30	2.69	3.41
	M	0.9758	2.79	2.69	3.42
S5	A	0.98	2.68	2.82	3.59
	D	0.9965	2.56	2.82	3.52
	M	0.9657	2.85	2.82	3.37
S6	A	0.9826	3.25	4.01	4.04
	D	0.997	3.49	4.01	4.46
	M	0.9881	3.93	4.01	4.14

Analysis of the data obtained revealed that even small structural changes can produce substantial differences in lipophilicity of derivatives investigated. The lowest lipophilicity was found for Aesculin (E2) Aesculetin (E1) and Umbelliferone (U) for which it seems clearly understandable due to presence of the highly polar glucose or two/one free hydroxylic groups respectively in their structure. Higher lipophilicity was exhibited by furanocoumarins. Introduction of the furan ring added ca. 2 lipophilicity units to the total lipophilicity. The highest value was measured for bergapten (B) – 2.76, next for isopimpinelin (I) – 2.46 and xantotoxine – 2.17. It is interesting is that bergapten and xantotoxine are both having one methoxy substituent but in different location (5' for bergapten and 9' for xantotoxin). Their lipophilicity differ by ca. 0.6 and isopimpinelin lipophilicity – 2.46 is in the middle between theirs. It would suggests that substituents in position 5 would increase lipophilicity when substituents in position 9 would lower it. Surprisingly also limettin exhibited lipophilicity at the bergapten level. Comparison of $mlogP_{EXP}$ values between limettine and scoparone gives ca. 1.5 units difference. Both compounds contain two methoxy groups. In their case position 5' seems also affecting the lipophilicity the most. Transfer of one methoxy moiety from position 5' to position 6' is responsible for such drastic decrease in lipophilicity (Table 2).

Table 2. Parameters of lipophilicity measurements for compounds investigated

	mean $logP_{EXP}$		r^2	R_{Mw}	$logP_{EXP}$	ΔlogP	S	φ_0
E1	0.65	A	0.9729	0.85	0.08	-0.57	2.08	0.4086
		D	0.9788	0.78	1.02	0.37	2.08	0.3774
		M	0.9885	1.18	0.86	0.21	2.23	0.5305
E2	0.00	A	0.9720	0.30	-0.78	-0.78	1.96	0.1531
		D	0.9725	0.21	0.41	0.41	1.63	0.1300
		M	0.9882	0.72	0.36	0.36	2.05	0.3532
U	0.83	A	0.9942	1.34	0.84	0.01	2.61	0.5134
		D	0.9725	0.21	0.41	-0.42	1.63	0.1300
		M	0.9838	1.52	1.24	0.41	2.38	0.6399
S	1.16	A	0.9444	1.13	0.51	-0.65	2.10	0.5381
		D	0.9781	0.92	1.17	0.01	1.87	0.4914
		M	0.9914	2.03	1.80	0.64	2.71	0.7506
L	2.76	A	0.9712	2.72	2.97	0.21	3.99	0.6817
		D	0.9891	1.99	2.33	-0.43	3.06	0.6503
		M	0.9846	3.10	2.98	0.22	3.71	0.8356
X	2.17	A	0.9738	2.15	2.09	-0.08	3.31	0.6496
		D	0.9799	1.62	1.93	-0.24	2.66	0.6105
		M	0.9883	2.65	2.48	0.31	3.31	0.8006
B	2.76	A	0.9576	2.56	2.72	-0.04	3.70	0.6919
		D	0.9894	2.05	2.39	-0.37	3.13	0.6550
		M	0.9833	3.27	3.17	0.41	3.78	0.8648
I	2.46	A	0.9614	2.30	2.32	-0.14	3.39	0.6785
		D	0.9836	1.82	2.14	-0.32	2.88	0.6326
		M	0.9782	3.05	2.92	0.46	3.69	0.8266

Table 3. Parameters of the correlations of chromatographic lipophilicity indices correlations (y = ax + b)

		$logP_{EXP}$	S	j0
A-D	a	0.5199	0.6856	0.9704
	b	0.8893	0.4645	-0.0231
	r2	0.989	0.9469	0.9964
A-M	a	0.7586	0.8411	0.9495
	b	0.957	0.5497	0.1871
	r2	0.9404	0.8762	0.9662
M-D	a	0.6505	0.7319	0.9905
	b	0.3024	0.2646	-0.1924
	r2	0.9473	0.8714	0.9688

All three solvent systems seems adequate for lipophilicity measuring of coumarins. Deviation of the results from the mean experimental lipophilicity ($mlogP_{EXP}$) is not higher than ± 0.5 for majority of cases. This statement is supported by good correlations of experimental lipophilicity $logP_{EXP}$ and chromatographic lipophilicity parameters S and φ_0. The results are presented in Table 3.

The results obtained are providing also very useful information concerning utility of the reversed-phase for separation of the coumarins in preparative way. Comparison of the S parameter (slope of the correlation curve) revealed that each coumarin investigated has different S value (Table 2). It confirms their different chemical character and different retention behavior. Those findings were the base of our idea of preparative isolation of coumarins on reversed-phase type stationary phases.

REFERENCES

1. Atta-ur-Rahman (Ed.): Studies in natural products chemistry. Vol. 32, Part L. Elsevier, Amsterdam, 2005, 515.
2. Borges F., Roleira F., Milhazes N. et al.: Curr. Med. Chem., 12, 887, 2005.
3. Boyce C.B.C., Milborrow B.V.: Nature, 208, 537, 1965.
4. Bruneton J.: Pharmacognosy, Phytochemistry, Medicinal Plants. Sec. Ed., Intercept Ltd., Hempshire, 1999, 263.
5. Colombaine M., Goll V., Muyart F. et al.: Planta Med., 67, 644, 2001.
6. Hansch C., Leo A., Hoekman D.: Exploring QSAR, ACS Professional Reference Book, Washington, 1995.
7. Kohlmunzer S.: Farmakognozja. PZWL, Warszawa, (2003).
8. Leo A., Hansch C., Elkins D.: Chem. Revs., 71, 525, 1971.
9. Matławska I.: Farmakognozja. Podręcznik dla studentów Farmacji. AM Poznań, 2005.
10. Matławska I., Bylka W., Drost-Karbowska K. et al.: Farmakognozja. Skrypt do ćwiczeń. AM Poznań, 1999.
11. Matosiuk D., Jóźwiak K.: J. Planar. Chromatogr., 13, 52, 2000.

12. Meisenberg G. M., Simmons W. H.: Principles of Medical Biochemistry. Mosby Inc., St. Louis, 1998.
13. Ojala T., Vuorela P., Kiviranta J. et al.: Planta Med., 65, 715, 1999.
14. Soczewiński E., Wachtmeister C.A.: J. Chromatogr., 7, 311, 1962.

SUMMARY

Coumarins are the class of natural compounds found in many medicinal plants with broad spectrum of activity. Many of them are also used as pharmaceutical substances, so there is an urgent need for efficient method of their isolation from the natural sources. Classical, extraction methods allow only isolation of the coumarin fraction, without separation of the elements of the mixture. It is caused mostly by coumarins small structural differences, affecting weekly their retention behavior. The parameter which ore directly can differentiate coumarins could be their lipophilicity. To confirm that, the measurement of the lipophilicity of the coumarin series by RP TLC method was performed in different eluent systems with standardization. Results confirmed great differences in lipophilicity of coumarins and also usefulness of the reversed-phase chromatography for separation of particular ones.

Keywords: natural coumarins, lipophilicity, partition coefficient, logP, RP-TLC, lipophilicity standards

STRESZCZENIE

Kumaryny są klasą naturalnych związków występujących w wielu roślinach leczniczych o bardzo szerokim spektrum działania. Wiele z nich znalazło zastosowanie także jako substancje farmaceutyczne, stąd konieczność opracowania wydajnych metod ich izolacji z surowców roślinnych. Klasyczne metody ekstrakcyjne pozwalają na wydzielenie jedynie frakcji kumarynowych, bez szczegółowego rozdziału mieszaniny. Wynika to z niewielkiego zróżnicowania strukturalnego kumaryn, co wpływa na ich retencję. Parametrem, który może znacznie lepiej różnicować kumaryny może być natomiast lipofilowość. Aby to potwierdzić zaplanowano badanie lipofilowości serii kumaryn metodą RP TLC w różnych układach elucyjnych ze standaryzacją. Wyniki potwierdziły duże różnice w lipofilowości kumaryn, a także przydatność metody chromatografii w układzie odwróconych faz dla ich rozdziału.

Słowa kluczowe: naturalne kumaryny, lipofilowość, współczynnik podziału, logP, RP-TLC, standard lipofilowości