ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2013, *58*(6), 527-532

In memoriam
Dr. Ing. Cornelia Uncuța and Dr. Filip Chiraleu

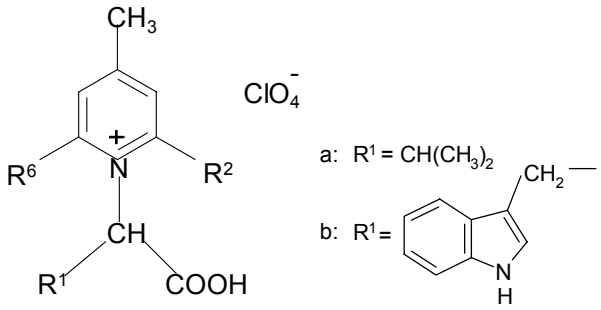
REACTION OF PYRYLIUM PERCHLORATES HAVING LONG ALKYL SUBSTITUENTS WITH AMINOACIDS**

Mariana Viorica BOGĂȚIAN,[a,*] Gheorghe BOGĂȚIAN,[b] Anca HÎRTOPEANU,[a] Călin DELEANU,[a,c] Alina NICOLESCU,[a,c] Maria MAGANU [a] and Petru FILIP [a]

[a] "Costin D. Nenitzescu" Centre of Organic Chemistry, Roumanian Academy,
202-B Spl. Independentei, Sect. 6, Box 35-108, RO-060023 Bucharest, Roumania
[b] S.B.S. Industrial Solutions, Biesterfeld Gruppe, 29-31 Aristide Pascal Str., Sect. 3, RO-031443, Bucharest, Roumania
[c] "Petru Poni" Institute of Macromolecular Chemistry, Group of Biospectroscopy,
41-A Grigore Ghica Voda Alley, RO-700487, Iași, Roumania

Received February 19, 2013

The reaction of 2,4,6-trisubstituted pyrylium salts having either one or two linear α-alkyl side chains **1**$_{1-4}$ with L-valine **2a** or L-tryptophan **2b** affording the corresponding pyridinium salts **3a**$_{1-4}$ or **3b**$_{1-4}$ is presented. All new compounds were characterized by ^{1}H- and ^{13}C-NMR, IR and elemental analysis.



INTRODUCTION

In the previous papers we described the preparation of new N-carboxypyridinium perchlorates obtained by direct reaction of the corresponding pyrylium salts having long alkyl substituents a with DL-alanine or DL-tyrosine.[1]

The synthesis of more pyrylium salts having different long alkyl substituents and corresponding pyridines and pyridinium salt was previously described in detail.[2,3a-e]

Currently in literature, there are no reports on the synthesis and specific properties of pyridinium salts having both long alkyl substituents on the heterocycle and the nitrogen substituted with an aminoacid moiety (L-tryptophan, L-valine). However there is an enormous amount of data on the applications of these two essential aminoacids. The most important within the past decade are presented here.

Balaban[4a-b] and Katritzky[4c-d] have reported previously the reaction of pyrylium salts with some α-aminoacids.

* Corresponding author: mbogat@cco.ro
** This is contribution no. 21 in the series "Pyrylium Salts with Long Alkyl Substituents". For some previous papers in this series see ref.[1, 2, 3a-e, 22-24]

Tryptophan is an essential amino acid which can be considered a derivative of alanine with an indole substituent on the β carbon. This induces new physico-chemical properties such as new hydrogen bonds.[5] L-Tryptophan (L-TRP) has a low solubility in water 11.4 g/L (25ºC) and it is slightly soluble in acetic acid. But it is soluble in hot water, hot alcohol and solutions of dilute acids and alkaline hydroxides. It is insoluble in diethyl ether and chloroform.[6] Thus, because of its slight solubility in water, TRP can be recovered from wastewaters only by adjusting the pH of the dilute solution.[7]

The solubility of some amino acids and two glycine peptides in aqueous ethanol and dioxane solutions and a hydrophobicity scale were established by Yasuhiko and Tandford.[8] There are many studies aiming to provide a rational explanation for the ability of various solvents to promote protein denaturation and protein movement. In the paper cited, solubility measurements in ethanol and dioxane serve a broader purpose, as they provide a semiquantitative estimate of interactions inside the native protein for hydrophobic moieties, in solvent relative to water.

Interfacial anchor properties of L-TRP residues in transmembrane peptides can dominate over hydrophobic matching effects in peptide-lipid interactions. These observations suggest that the lipid adaptations are not primarily directed to avoid a peptide-lipid hydrophobic mismatch, but instead to prevent displacement of the tryptophan side chains from the polar-apolar interface.[9]

There are five major classes of plant growth regulators (PGRs), including auxins which play a vital role in controlling plant growth.[10] Sarwar & Frankenberger (1994); Zahir *et al*. (2000, 2005), Khalid *et al*. (2006) reported the mechanism of action of TRP on plant growth.[11a-c] Because TRP is a physiological precursor of auxins, the study described by Zahir *et al.* [12] was conducted to evaluate the potential of L-TRP dependent biosynthesis of auxins by *Rhizobium* for increasing growth and yield of mung bean (*Vigna radiata* L.)

Recently, application of gene targeting to designed mutation breeding of high-tryptophan rice were described. Useful informations applied directly to molecular breeding, including metabolic engineering were reported.[13]

Tryptophan and its derivatives are known to play important biological roles and tryptophan metabolism within different tissues is associated with numerous physiological functions. The liver regulates tryptophan homeostasis by degrading excess tryptophan. L-TRP degradation into kynurenine by immune cells plays a crucial role in the regulation of immune response during infections, inflammations and pregnancy. Serotonin is synthesized from tryptophan in the gut and also in the brain, where tryptophan availability is known to influence the sensitivity to mood disorders.[14]

Tyrosine and/or tryptophan containing peptides are very effective antioxidants. They can be used for therapy and prevention of diseases that involve oxidative processes in the extracellular space. Such diseases include arteriosclerosis, cataracts, diabetes, arthritis as well as ageing of skin and joints. The antioxidative peptides developed contain aromatic systems, which are able to form stable radicals, especially tyrosine and tryptophan residues.[15]

Valine was isolated by Emil Fischer from casein back in 1901. Presently, valine is known as one of the three branched-chain essential amino acids, which provides a stimulative activity for the nervous system and cognitive function. Valine is soluble in cold water and is sparingly soluble in methanol or acetone. This amino acid cannot be produced by the human body and must be obtained from food or supplements. Valine is important for everyday body functions and for maintaining musculature, as well as for the regulation of the immune system. This particular amino acid is not processed by the liver, but is taken up by muscles. It can be obtained from kidney beans, leafy vegetables, poultry and milk.[16a,b] We have previously monitored valine concentrations in urine.[17a,b] Some derivatives of valine have antibiotic action. Valine is also a precursor in the penicillin biosynthetic pathway and it is known to inhibit the transport of tryptophan across the blood-brain barrier.[18] Particular symptoms of valine deficiency include susceptibility to allergens.[19] Valine can be used to treat amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease) and a genetic disorder called McArdle's disease.[20]

The present paper describes the synthesis of new N-carboxy-2,4,6-trisubstituted pyridinium perchlorates having two various linear long side chains in 2,6 positions. Having supplementary residues derived from L-valine or L-tryptophan, these compounds are expected to have biological activity and to be useful as efficient vectors in nonviral gene therapy.

RESULTS AND DISCUSSION

In previous papers[2,3a-e,21-24] we have reported the synthesis of a series of pyrylium salts with long alkyl substituents in α or γ. positions and derived pyridines and pyridinium salts. Optimised preparation conditions and some of their applications were presented in detail.[21]

Two methods were employed for conversion into pyridinium salts: either the direct reaction of pyrylium salts with amines, in boiling alcohol, or the reaction optimized by Katritzky at room temperature in methylene chloride.[25]

The present paper describes the preparation of a new class of heterocyclic compounds obtained by direct reaction of the corresponding pyrylium salts having long alkyl substituents $\mathbf{1_{1\text{-}4a}}$ with essential amino acids L-valine (**2a**) or L-tryptophan (**2b**).

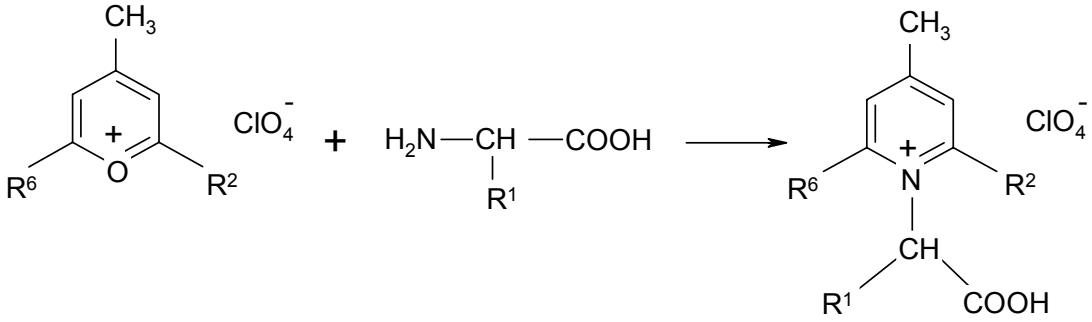
Our first attempt to synthesize these new compounds is summarized in Scheme 1.

Table 1 presents the new compounds described in this paper.

Balaban (glycyl-glycine,β-alanine)[4a-b] and Katritzky (glycine, lisine, glycyl-glycine)[4c-d] have previously reported the reaction of pyrylium salts (trimethyl- or triphenylpyrylium perchlorate) with the cited β -aminoacids. Depending on the reaction conditions good yields in pyridinium salts may be obtained *vs* decyclization products. Also the basicity of amines or aminoacids may play an important role.[26]

We have previously reported that the high solubility differences between pyrylium perchlorates having long alkyl substituents in α positions and α -aminoacids as DL-alanine dictated other reaction conditions.[1]

As previously shown,[1,2,3a-e,22-24] 2,4,6-tri- or 2,3,4,6-tetraalkylpyrylium perchlorates with one or two long alkyl substituents in 2,6 positions synthesised in our laboratory present a good solubility in alcohols, DMF (dimethylformamide), CH_2Cl_2 and low solubility in diethyl ether and water. All these compounds have relative low melting points (m.p.) at temperatures ranging between 60 and 90°C.



Scheme 1 – Synthesis of N-carboxypyridinium perchlorates.

Table 1

Newly synthesized compounds **3a-b**

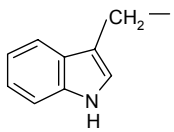
Cpd.	R^1	R^2	R^6
3a$_1$	—CH(CH$_3$)$_2$	$C_{11}H_{23}$	CH_3
3a$_2$		$C_{11}H_{23}$	$C_{11}H_{23}$
3a$_3$		$C_{15}H_{31}$	$C_{15}H_{31}$
3a$_4$		$C_{17}H_{35}$	$C_{17}H_{35}$
3b$_1$		$C_{11}H_{23}$	CH_3
3b$_2$		$C_{11}H_{23}$	$C_{11}H_{23}$
3b$_3$		$C_{15}H_{31}$	$C_{15}H_{31}$
3b$_4$		$C_{17}H_{35}$	$C_{17}H_{35}$

Table 2

Selected signal assignments for ^{1}H- and ^{13}C - NMR spectra of compounds **3a$_{1-4}$** and **3b$_{1-4}$**

Cpd.	**^{1}H / ^{13}C chemical shifts δ (ppm) in $CDCl_3$ Aromatic ring**											
	2C	3C	4C	5C	6C	NH	C^2	C^3	C^4	C^5	C^6	C^7
3a$_1$	-/ 155.62	7.53/ 127.90	-/ 159.08	7.67/ 130.17	-/ 158.36.	-	-	-	-	-	-	-
3a$_2$	-/ 158.78	-/-	-/ 159.15	7.59/ 129.93	-/-	-	-	-	-	-	-	
3a$_3$	-/ 158.77	-/-	-/ 159.14	7.57/ 129.91	-/-	-	-	-	-	-	-	-
3a$_4$	-/ 158.76	-/-	-/ 159.12	7.55/ 129.90	-/-	-	-	-	-	-	-	-
3b$_1$	-/ 155.14	7.37/ 128.44	-/ 159.26	7.49/ 129.32	-/ 158.66	9.02/ -	7.30/ 124.31	-/ 112.30	7.44/ 117.02	7.05/ 119.75	7.11/ 119.88	7.38/ 122.35
3b$_1$	-/ 158.50	-/-	-/ 159.90	7.54/ 126.13	-/-	9.02/ -	7.37/ 127.06	-/ 112.22	7.47/ 116.88	7.08/ 119.91	7.11/ 119.78	7.39/ 126.67
3b$_3$	-/ 158.49	-/-	-/ 159.88	7.53/ 129.10	-/-	9.02/ -	7.38/ 127.09	-/ 112.24	7.48/ 116.89	7.09/ 119.92	7.11/ 119.76	7.39/ 126.68
3b$_4$	-/ 158.49	-/-	-/ 159.85	7.51/ 129.08	-/-	9.02/ -	7.39/ 127.10	-/ 112.25	7.48/ 116.89	7.09/ 119.92	7.11/ 119.76	7.39/ 126.68

Cpd.	**^{1}H / ^{13}C chemical shifts δ (ppm) in $CDCl_3$ Aliphatic substituents**													
	$4CH_3$	$6CH_3$	$1'CH_2$	$2'CH_2$	$3'CH_2$	(n'-2) CH_2^*	(n'-1) CH_2	n' CH_3	1" CH	2" CH	2" CH_2	n''$_1$ CH_3	n''$_2$ CH_3	COOH
3a$_1$	2.59/ 21.67	2.75/ 21.43	3.02/ 35.54	1.82/ 32.79	1.64/ 30.06	-/ 33.74	-/ 22.60	0.88/ 14.11	5.04/ 70.65	2.87/ 31.92	-	1.26/ 18.58	1.26/ 10.04	9.76/ 169.43
3a$_2$	2.35/ 21.23	-/-	3.09/ 33.85	1.80/ 31.95	1.63/ 29.70	-/ 33.72	-/ 22.70	0.88/ 14.11	4.84/ 67.54/	2.97/ 29.74		1.26/ 18.49	1.26/ 10.01	9.77/ 169.13
3a$_3$	2.36/ 21.24	-/-	3.11/ 33.87	1.80/ 31.97	1.64/ 29.69	-/ 33.71	-/ 22.70	0.88/ 14.11	4.80/ 67.50	2.98/ 29.72		1.26/ 18.45	1.26/ 10.01	9.77/ 169.13
3a$_4$	2.36/ 21.24	-/-	3.11/ 33.87	1.80/ 31.97	1.64/ 29.67	-/ 33.69	-/ 22.70	0.88/ 14.11	4.78/ 67.48	2.99/ 29.70		1.26/ 18.45	1.26/ 10.01	9.77/ 169.13
3b$_1$	2.40/ 21.55	2.85/ 21.14	3.38/ 33.85	1.92/ 31.92	1.62/ 30.32	-/ 33.73	-/ 22.01	0.88/ 14.12	5.58/ 67.14	-/-	4.09/ 33.98	-/-	-/-	9.14/ 178.89
3b$_2$	2.37/ 21.23	-/-	3.06/ 31.95	1.96/ 30.47	1.64/ 29.70	-/ 31.70	-/ 22.69	0.87/ 14.10	5.62/ 67.09	-/-	4.32/ 33.75	-/-	-/-	9.09/ 177.99
3b$_3$	2.35/ 21.20	-/-	3.04/ 31.93	1.97/ 30.49	1.64/ 29.71	-/ 31.71	-/ 22.70	0.87/ 14.10	5.64/ 67.11	-/-	4.33/ 33.79	-/-	-/-	9.09/ 177.99
3b$_4$	2.33/ 21.17	-/-	3.03/ 31.90	1.97/ 30.48	1.64/ 29.71	-/ 31.72	-/ 22.70	0.87/ 14.10	5.65/ 67.13	-/-	4.33/ 33.79	-/-	-/-	9.09/ 177.99

In a previous paper[1] we reported the reaction of pyrylium salts having long alkyl substituents with DL-alanine, which is characterized by a medium or low solubility in usual solvents. The corresponding pyridinium perchlorates were obtained with good yields by refluxing the reagents in a water-ethanol mixture only containing a small amount of sodium lauryl sulphate (SLS). In these cases, the use of the anionic surfactant increased system's miscibility and favoured the mixing of reagents.

For the cases presented in this paper, because L-TRP has a good solubility in hot water and hot alcohol[6] and L-valine[16] is soluble in cold water, the addition of SLS was not necessary.

By employing usual conditions (excess of aminoacids in water-ethanolic solution) the corresponding pyridinium perchlorates were obtained. The progress of the reaction was monitored by TLC.

After TLC separation, the purity for all new compounds was confirmed by ^{1}H, ^{13}C-NMR, IR spectra and elemental analysis. The results are presented in the Experimental part.

Similar results are obtained for the reaction of pyrylium perchlorates having different long alkyl substituents in α positions with tyrosine.[1]

Our results confirm that, in the synthesis of N-carboxypyridinium salts with different long alkyl substituents from the corresponding pyrylium salts, the difference between the solubilities of the reagents have a crucial role on the yields.

The ^{1}H- and ^{13}C-NMR chemical shifts of these compounds were in agreement with the previously reported NMR data.[1,3a-e,23,24] The chemical shift assignments in the ^{1}H- and ^{13}C-NMR spectra were confirmed by 2D experiments (COSY, HMQC and HMBC). Practically all individual signals could be assigned in the ^{13}C-NMR spectra.

Table 2 presents the ^{1}H- and ^{13}C-NMR chemical shifts for these new compounds. The carbon atom numbering for compounds **3b$_{1-4}$** is shown in Fig. 1.

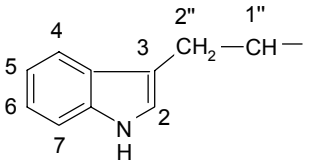


Fig. 1 – The atom numbering for compounds **3b$_{1-4}$**.

The IR spectra for some representative compounds **3a$_{1,2}$** and **3b$_{1,2}$** are presented in corresponding experimental part. For all new compounds presented here **3a$_{1-4}$** and **3b$_{1-4}$**, the IR spectra were in full agreement with those of the 2,4,6-tri- or 2,3,4,6-tetrasubstituted homologues described earlier.[1]

The UV-Vis spectra and CMC determinations will be presented elsewhere.

EXPERIMENTAL

The NMR spectra have been recorded on BRUKER AVANCE DRX 400 and AVANCE III 400 instruments, equipped with 5 mm both inverse and direct detection multinuclear probeheads and field gradients on the z axis, operating at 400.13 MHz for ^{1}H and at 100.61 MHz for ^{13}C nuclei. The COSY45, HMQC and HMBC spectra have been acquired with standard Bruker parameters in the versions employing pulsed field gradients. All spectra have been recorded in deuterated chloroform, and the chemical shifts have been reported as δ values referenced to TMS as internal standard. Infrared spectra were recorded on a BRUKER VERTEX 70 instrument equipped with a Golden Gate diamond ATR. Melting points were measured in open capillary tubes (for the low melting compounds) or on a hot-stage melting points apparatus (equipped with a polarizer to check for nematic properties).

Synthesis of N-carboxypyridinium salts

A mixture of pyrylium salt **1$_{1-4}$** (0.1-0.2 mmoles) and L(+) valine **2a** (0.3-0.4 mmoles) or L-tryptophan **2b** (0.5 - 0.8 mmoles) was suspended in a water-ethanol solution (EtOH:H_2O 3:1) and heated at gentle reflux for 10 hours when the colour turned to dark orange or cognac. After cooling, an opalescent dispersion was obtained for all compounds.

In the case of compounds **3a$_{1-4}$** the reaction mixture was evaporated and concentrated in vacuum. The residue was dissolved in 100 mL CH_2Cl_2 and extracted with a mixture water :$HClO_4$ = 12:1 (3x50 mL, threefold, at 25^{o}C). The combined organic layers were washed with 300 mL water (at 25^{o}C), dried ($MgSO_4$) and concentrated under reduced pressure. The pyridinium salts were separated by thin layer chromatography (silica gel Merck type 60G, solvents ethyl acetate: methanol = 3:5).

Compounds **3a$_{1-4}$** are waxy oils which crystallized in the refrigerator and have m.p. under 50^{o}C (**3a$_1$**: 28-28.5^{o}C, **3a$_2$**: 22-24^{o}C and under 20 ^{o}C for **3a$_{1-2}$**).

Yields: 55% (**3a$_1$**), 53% (**3a$_2$**), 50% (**3a$_3$**), 48% (**3a$_4$**).

Elemental analyses for compounds **3a$_{1-6}$**:

3a$_1$ **$C_{23}H_{40}NClO_6$** Calcd: N 3.03; Found N 3.04;
3a$_2$ **$C_{33}H_{60}NClO_6$** Calcd: N 2.33; Found N 2.32;
3a$_3$ **$C_{41}H_{76}NClO_6$** Calcd: N 1.96; Found N 1.97;
3a$_4$ **$C_{45}H_{84}NClO_6$** Calcd: N 1.82; Found N 1.84.

IR spectra, ν (cm^{-1}):

3a$_1$: 3464.72, 2955.26, 2922.19, 2852.85, 2033.64, 1973.86, 1736.60, 1635.71, 1573.12, 1464.24, 1376.19, 1261.30, 1215.43, 1092.00, 928.86, 871.15, 720.65, 622.34;

3a$_2$: 3460.01, 2958.85, 2919.55, 2851.04, 2168.18, 2023.11, 1967.05, 1711.77, 1635,13, 1465.10, 1412.47, 1377.43, 1259.22, 1087.20, 1014.31, 865.41, 794.65, 721.90, 623.39.

In the case of compounds **3b$_{1-4}$** the reaction mixture was evaporated to dryness or concentrated in vacuum to half volume. The residue was dissolved in 100 mL $CHCl_3$ and extracted with a mixture water: HCl = 12:1 (3x50 mL, threefold, at 25ºC). To the combined organic extracts $HClO_4$

was added, the mixture was then washed with 300 mL water (at 25ºC), dried ($MgSO_4$) and concentrated. The pyridinium salts were separated by thin layer chromatography (silica gel Merck type 60G, solvents ethyl acetate: acetic acid: methanol = 6: 3:1).
Yields: 48% (**3b$_1$**), 44% (**3b$_2$**), 44% (**3b$_3$**), 46% (**3b$_4$**).
Elemental analyses for compounds **3b$_{1-4}$**:
3b$_1$ $C_{29}H_{43}N_2ClO_6$ Calcd: N 5.09; Found N 5.07;
3b$_2$ $C_{39}H_{63}N_2ClO_6$ Calcd: N 4.05; Found N 4.06;
3b$_3$ $C_{47}H_{79}N_2ClO_6$ Calcd: N 3.49; Found N 3.50;
3b$_4$ $C_{51}H_{87}N_2ClO_6$ Calcd: N 3.26; Found N 3.25.
IR spectra, ν (cm^{-1}):
3b$_1$: 3383.89, 2949.61, 2921.61, 2852.47, 2094.38, 1729.46, 1635.19, 1572.38, 1460.60, 1377.78, 1261.96, 1094.98, 928.35, 856.76, 743.92, 721.56, 622.09 for
3b$_2$: 3397.86, 2957.23, 2919.76, 2851.97, 2197.97, 2140.29, 1713.34, 1636.85, 1614.45, 1573.81, 1466.97, 1379.34, 1263.25, 1101.32, 887.63, 744.17, 723.51, 624.88.

Acknowledgements: This work was supported by a grant of the Ministry of National Education and Research through the Roumanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-ID-PCCE-2011-2-0028.

REFERENCES

1. M. V. Bogăţian, G. Bogăţian, A. Hirtopeanu, C. Deleanu, A. Nicolescu, M. Maganu and P. Filip, *Rev. Roum. Chim.*, **2011**, *56*, 325.
2. M. V. Bogăţian, in "Săruri de piriliu şi piridiniu având substituenţi alchilici lungi", Editura Vidia, Bucureşti, 2010.
3. a) M. V. Bogăţian, A. Hirtopeanu, A. Nicolescu, G. Bogăţian, C. Deleanu, M. Maganu and P. Filip, *Rev. Roum. Chim.*, **2009**, *54*, 927; b) M. V. Bogăţian, S. Serban, G. Bogăţian, A. Hirtopeanu, C. Deleanu and P. Filip, *Rev. Roum. Chim.*, **2010**, *55*, 277; c) M. V. Bogăţian, M. Vinătoru, G. Bogăţian, F. Chiraleu, M. Godeanu and C. Deleanu, *Rev. Roum. Chim.*, **2008**, *53*, 813; d) M. V. Bogăţian, C. Deleanu, D. Simion (Nuţă), M. Maganu, A. C. Corbu, F. Chiraleu and G. Bogăţian, Rev. *Roum. Chim*., **2006**, *51*, 273; e) M. V. Bogăţian, M. Vinatoru, G. Bogătian, G. Mihai and T. S. Balaban, *Rev. Roum. Chim*., **2004**, *49*, 819.
4. a) A.T. Balaban, A. Dinculescu, G. N. Dorofeenko, G. W. Fischer, A. V. Kobilik, V. V. Mezheritskii and W. Scroth, "Pyrylium Salts. Syntheses, Reactions and Physical Properties", Adv. Heterocyclic Chem., Suppl. Vol. 2, A. R.. Katritzky, Ed. Academic Press, New-York, **1984**; b) C. Toma, A. T. Balaban, *Tetrahedron*, **1966**, *22*, 27; c) A. R. Katritzky, N. E. Grzeskowiak, N. F. Eweiss and E. A. Elsherbini, *J. Chem. Soc.*, *Perkin Trans. 1*, **1983**, 497; d) A. R. Katritzky, *Tetrahedron*, **1980**, *36*, 679.
5. E. Bratland, N. F. Magitta, A.S. Boe Wolff, T. Ekern, P. M. Knappskog, O. Kampe, J. Haavrik and E. S. Husebye, *Immunobiology*, **2012**, *2985*, 516.
6. S. Verheeyden, A. Sillen, A. Gils, P. J. Declercj and Y. Engelborghs, *J. Biophys.*, **2003**, *85*, 501.
7. L. F. Liu, L. L. Yang. K. Y. Jin, D. Q. Xu, and C. J. Gao, *Sep. Purif. Technol*., **2009**, *66*, 443.
8. N. Yasuhiko and C. Tandford, *J. Biol. Chem.*, **1971**, *246*, 2211.
9. M. R. R. de Planque, B. B Bonev, J. A. A. Demmers, D. V. Greathouse, R. E. Koeppe, F. Separovic, A Watts and J. A. Killian, *Biochemistry*, **2003**, *42*, 5341.
10. W. T. Frankenberger Jr. and M. Arshad, in "Phytohormones in Soil: Microbial production and Function", Marcel Dekker, New York, 1995.
11. a) M. Sarwar and W. T. Frankenberger, *J. Plant Soil*, **1994**, *160*, 97; b) Z. A. Zahir, M. A. R. Malik and M. Arshad, *Pak. J. Biol. Sci*., **2000**, *3*, 289; c) A. Khalid, M. Arshad and Z. A. Zahir in "Biological Approaches to Sustainable Soil Systems", Eds. N. Uphoff , A. S. Ball, E. Fernandes, H. Herren, O. Husson, M. Laing, C. Palm, J. Pretty, P. Sanchez, N. Sanginga and J. Thies. Taylor & Francis/CRC, Boca Raton, Florida, 2006.
12. Z. A. Zahir, H. M. Yasin, M. Naveed, M. A. Anjum and M. Khalid, *Pak. J. Bot*., **2010**, *42*, 1771.
13. H. Saika, A. Oikawa, F. Matsuda, H. Onoreda, K. Saito and S. Toki, *Plant Physiology*, **2011**, *156*, 1269.
14. N. Le Floc'h, W. Otten and E. Merlot, *Amino Acids*, ***2011***, *41*, 1195.
15. G. Huether, W. Kochen, T. J. Simat, H. Steinhart, *Adv. Exp. Med. Biol.*, **1999**, 467.
16. a) M. A. Kumar, A. R. Bitla, K. V. Raju, S. M. Manohar, V. S. Kumar and S. R. Narasimba, *Saudi J. Kidney Dis. Transpl.*, **2012**, *23*, 1202; b) P. C. Calder, *J. Nutr.*, **2006**, *136*, 288.
17. a) C. Ciurtin, A. Nicolescu, L.-I. Stefan, E. Kovacs, I. C. P. Smith and C. Deleanu, *Rev. Chim. (Bucharest)*, **2007**, *52*, 51; b) L. I. Stefan, A. Nicolescu, S. Popa, M. Mota, E. Kovacs and C. Deleanu, *Rev. Roum. Chim.*, **2010**, *55*, 1033.
18. A. Rosenberg, in "The structure of biological science", Cambridge Univ. Press., Cambridge, **1985**.
19. E. Kakazu, N. Kanno, Y. Ueno and T. Shimosegawa, *J. Immunology*, **2007**, *179*, 7137.
20. a) L. S. Eriksson, A. Persson and J. Wahren, *Gut*, **1982**, *23*, 801; b) G. Marchesini, G. Bianchi, M. Merli, P. Amodio, C. Panella, C. Loguercio, F. Rossi Fanelli and R. Abbiati, *Gastroenterology*, **2003**, *124*, 1792.
21. M. V. Bogăţian, "Membrane", Editura Vidia, Bucureşti, **2011**.
22. M. V. Bogăţian, *Ph. D. Thesis*, Roumanian Academy, "Costin D. Nenitzescu" Centre of Organic Chemistry, Bucharest, **1998**.
23. a) M. V. Bogăţian, A. Hîrtopeanu, A. Nicolescu, F. Chiraleu, M. Maganu, M. Vinătoru, D. Simion and C. Deleanu, *Rev. Roum. Chim*., **2008**, *53*, 431; b) M. V. Bogăţian, M. Vinătoru, G. Bogăţian, D. Nuţă, F. Chiraleu, V. Câmpeanu and C. Deleanu, *Rev. Roum. Chim*., **2007**, *52*, 899; c) M. V. Bogăţian, V. Câmpeanu, C. Deleanu, A. C. Corbu, G. Bogăţian and T. S. Balaban, ARKIVOC, **2005**, *(x)*, 272. (http://www.arkat-usa.org/ark/journal/2005/I10_Balaban/1275/AB-1275B.pdf); d) M. V. Bogăţian, C. Deleanu, S. Udrea, F. Chiraleu, M. Plăveţi, M. G. Dănilă, G. Bogăţian and T. S. Balaban, *Rev. Roum. Chim.*, **2003**, *48*, 712.
24. a) M. V. Bogăţian, V. Câmpeanu, S. Serban, G. Mihai and T. S. Balaban, *Rev. Roum. Chim.*, **2001**, *46*, 115; b) M. V. Bogăţian, G. Mihai, M. Plăveţi, F.Chiraleu, C. Deleanu, V. Bădescu and T. S. Balaban, *Rev. Roum. Chim.*, **1996**, *41*, 979; c) M. V. Bogăţian, C. Deleanu, G. Mihai and T. S. Balaban, *Z. Naturforsch.*, **1992**, *47b*, 1011; d) T. S. Balaban, M. V. Bogăţian, M. Plăveţi, V. Bădescu and G. Mihai, *Rev. Roum. Chim.*, **1991**, *36*, 229.
25. A. R. Katritzky and P. C. Patel, *J. Chem. Soc. Perkin Trans. I*, **1982**, 117.
26. A. Dinculescu and A. T. Balaban, *Rev. Roum. Chim*., **1980**, *25*, 1505.