ELSEVIER

Thermochimica Acta 381 (2002) 19–29

thermochimica acta

www.elsevier.com/locate/tca

Thermal and pharmacological characterization of some new bis-xanthine derivatives

Lenuta Profire, Gina Gabriela Bumbu, M. Costuleanu, Gh. Danila, C. Vasile*

P. Poni Institute of Macromolecular Chemistry, Gr. Ghica Voda Alley, 41 A, 6600 Iasi, Romania

Received 18 January 2001; accepted 11 June 2001

Abstract

The 8-substituted bis-xanthine derivatives with various substituents have been synthesized taking in the view the previous results with xanthine derivatives, exhibiting enhanced and multiplied pharmacological properties in respect with those of theophylline. Their chemical structures have been assessed by IR, UV and ^{1}H NMR spectroscopy and elemental analysis.

The influence of the non-cyclic and cyclic substituents on thermal and thermo-oxidative behaviors has been followed by thermogravimetry and differential scanning calorimetry. These methods are useful to determine thermal stability, the purity of the compounds and crystallinity. The pharmacological tests have proved that their action both on bronchopulmonary and cardiovascular systems depends on the nature of the substituent.

Keywords: Xanthine; Theophylline; Anti-inflammatory activity

1. Introduction

The benefit of the cardiovascular and bronchopulmonar effects of theophylline is well-known [1–4]. However, it also presents some serious adverse effects because of the absence of selectivity of its action [5].

In our previous papers [6–8] the thermal and pharmaceutical characterization of some new xanthine derivatives has been presented. Supposing that double functionality will impart higher activity and in order to enhance the cardiovascular and bronchopulmonar effects with selective action and also to reduce the serious adverse effects which appears at high doses of theophylline, new bis-xanthine derivatives have been synthesized.

The characterization of these new product implies both the determination of their structure and physicochemical properties and also carefully testing of the pharmacological activity.

This paper deals with the synthesis and thermal characterization of the 8-substituted bis-xanthine derivatives containing various substituents comparatively with starting materials and intermediary products of synthesis namely theophylline and 7-(2,3-epoxy-propyl)-theophylline, respectively. Their pharmacological activity has also been tested.

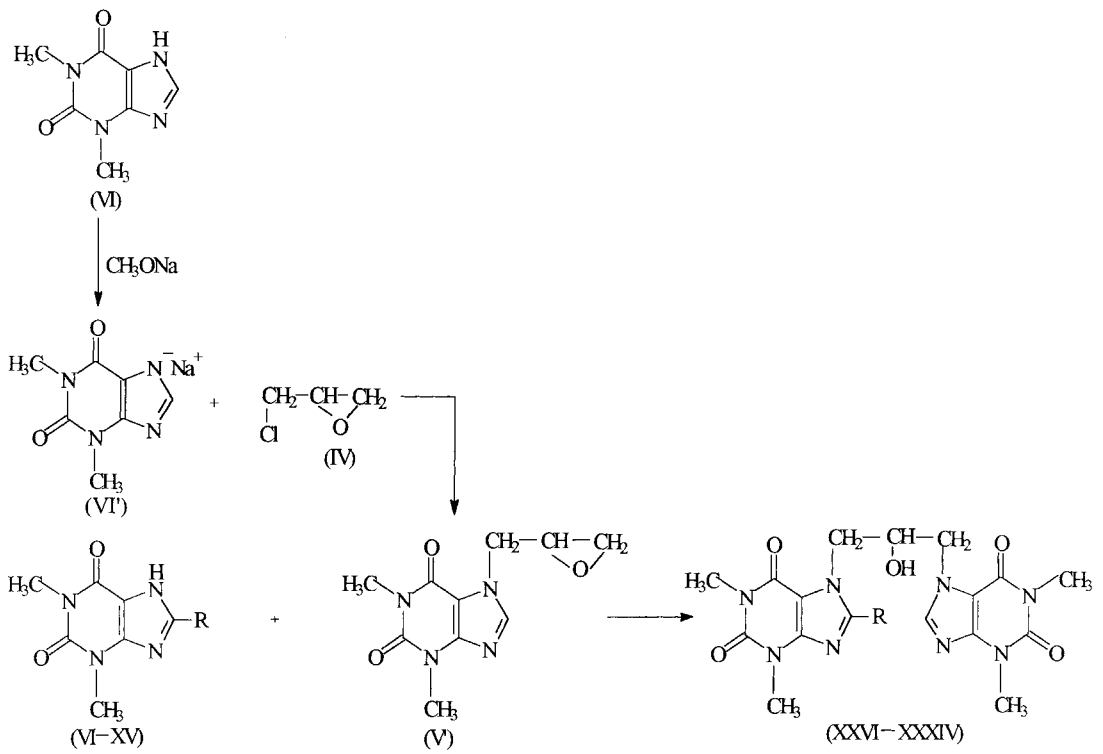
2. Experimental

2.1. Synthesis of bis-xanthine derivatives

The synthesis of 8-substituted bis-xanthine derivatives was performed in three steps (Scheme 1). In the first step, the theophylline (**VI**) was turned in to the

* Corresponding author. Present address: Gr. T. Popa University of Medicine and Pharmacy, Iasi, Romania. Tel.: +40-3214-4909; fax: +40-3221-1299.
E-mail address: cvasile@ichpp.tuiasi.ro (C. Vasile).

0040-6031/02/$ – see front matter
PII: S0040-6031(01)00639-6



Scheme 1.

sodium salt (**VI′**) in medium alkaline, that further reacts with epichlorhydrine (**IV**), the second step, in the conditions described in [9] to form 7-(2,3-epoxy-propyl)-theophylline (**V′**). In the third step, the 7-(2,3-epoxy-propyl)-theophylline (**V′**) reacts in mild conditions at the boiling temperature of the ethyl alcohol with 8-substituted theophylline giving the bis-xanthine derivatives (**XXVI–XXXIV**).

Nine derivatives with various substituents in 8-position have been obtained. The R-substituent type codes for various 8-R-bis-xanthine derivatives are given in the first column of the Table 1. All reagents used in synthesis were of analytical purity.

The synthesized bis-xanthine derivatives have been purified by repeated crystallizations from various organic solvents. All compounds are white crystalline solids at room temperature. The control of purity was achieved by melting point determination using Boetius microscope and differential scanning calorimetry (DSC). Supplementary peaks in DSC curves or large endothermic peaks evidenced the presence of impurities.

2.2. Characterization of bis-xanthine derivatives

2.2.1. Spectral methods

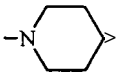
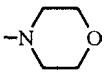
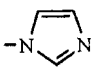
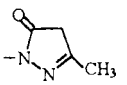
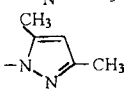
Each compound was characterized by elemental analysis, IR, UV and ^{1}H NMR spectroscopy. IR spectra were recorded on a 577 Perkin Elmer spectrophotometer. UV spectra were recorded with a HP 8453 Hewlett-Packard UV–VIS spectrophotometer. ^{1}H NMR spectra were recorded using a JEOL C-60 spectrometer at 60 MHz on DMSO (d_5) or CCl_4 solutions.

2.2.2. Thermoanalytical methods

In pharmaceutical industry, thermoanalytical methods play an important role either in the development of new products and their characterization or as a simple quality control tool [10–13]. Two methods have been applied for study of the bis-xanthine derivatives: DSC and thermogravimetry (TG).

DSC curves were recorded by means of the Mettler DSC 12E instrument under the following conditions: heating rate 10 °C/min, nitrogen flow 50 ml/min.

Table 1
UV spectral data for bis-xanthine derivatives

Compound	R	UV absorption maximum (nm)
XXVI	H	277
XXVII	Br	278
XXVIII	NO_2	277
XXXIV		281
XXIX		277
XXX		283
XXXI		278
XXXII		278
XXXIII		278
VI	Theophylline	278

Mass of sample 1.2–6 mg; investigated temperature range 20–300 °C. Calibration of the calorimeter was performed by determining the heat of fusion of indium. Melting point of indium is 156.6 °C, $\Delta H_f = 28.43$ J/g [14]. Each experiment was repeated at least two times. The baseline of DSC curves, for delimitation of the area of melting peak, was obtained by interpolation of initial and final straight line.

The TG and differential thermogravimetric (DTG) curves were recorded on a Paulik–Paulik–Erdey type derivatograph, MOM, Budapest in the following conditions: heating rate 12 °C/min, sample mass 50 mg, air flow 30 ml/min. In both kinds of thermoanalytical experiments, the sample was in powder form.

3. Results and discussion

3.1. Spectral characterization of the bis-xanthine derivatives

The representative IR spectra of the bis-xanthine derivatives are presented in Fig. 1 and the characteristic IR bands (Table 2), maxima of absorption in UV spectra (Table 1) and chemical shifts in ^{1}H NMR spectra (Table 3) are given in Tables 1–3, respectively, their assignment was made according to the literature data [4]. The ^{1}H NMR signals corresponding to aromatic protons are modified, Table 3, after substituent bonding. The number of protons is reduced and also, the number of signals. Particular spectral characteristics for each compound have been found; therefore the substituent introduction is proven.

The spectral characteristics and calculated formula according to elemental analysis results, Table 4 are in a good agreement with the expected structures proposed in the Scheme 1.

3.2. Properties

As it is expected, the changes in formula determine the change in the properties such as the solubility Table 5, thermal properties Table 6, and pharmacological activity Table 7.

All compounds are soluble in very polar solvents such as dimethylformamide (DMF), dimethylsulfoxide (DMSO), alcohols and acetone and insoluble in aromatic non-polar solvents such as benzene and diethylether. In water, are only partial soluble compounds with substituents with low volume. The substituent significantly affects the thermal behavior, Figs. 2 and 3 and Table 6.

The starting substances for synthesis of bis-xanthine derivatives exhibits melting temperatures of 274.3 and 270 °C, Fig. 2 and Table 6 and melting heats of 23.7 and 28.9 kJ/mol, respectively. As it was shown in the previous paper [6], the theophylline melts with decomposition and the xanthine (**XXVI**) melts at an intermediary temperature of 204 °C. The melting temperatures and heats are also specific for each bis-xanthine derivatives.

The bis-xanthine derivatives were classified in two groups according to the nature of substituent: namely, derivatives containing non-cyclic or cyclic substituents. Each group exhibits particular DSC and TG curves, Figs. 2 and 3, respectively. The 8-bromo (**XXVII**) and nitro (**XXVIII**) bis-xanthine derivatives have melting point of 224 and 275 °C, respectively, and different melting heats of ∼28 and 50.8 kJ/mol, respectively, the first close to that of the theophylline and bis-xanthine while the second bis-xanthine

derivative exhibits higher melting temperature and heat than that of xanthine (**XIV**), starting materials, therefore their structure is different.

Most of the 8-substituted bis-xanthine derivatives with heterocyclic substituents containing only carbon and nitrogen (**XXIX**, **XXX**, **XXXI**, **XXXII**, **XXXIII**) have particular melting temperature most of them having lower melting temperature than starting materials, except the compound **XXXI** with a high melting temperature at 285 °C but its melting is accompanied by oxidation as appears from overlapped exothermic peak with the melting, as appears also from comparison of the values of T_m and T_i from the Table 6, melting heat being placed above onset temperature of decomposition. The derivative with a heterocyclic substituent containing carbon, nitrogen and oxygen exhibits both the lowest melting point of 227 °C and a melting heat only 11.3 kJ/mol. The TG results are given in Fig. 3 and Table 6.

Generally, the starting compounds decompose in a single step (Fig. 3a) occurring in the 226–500 °C temperature range with a mass loss of 80–85 wt.%. Bis-xanthine derivatives (**XXVIII**, **XXX** and **XXXI**) also decompose in a single step in this temperature range, but the DTG peaks of the last two compounds exhibit many inflexions because of the particular

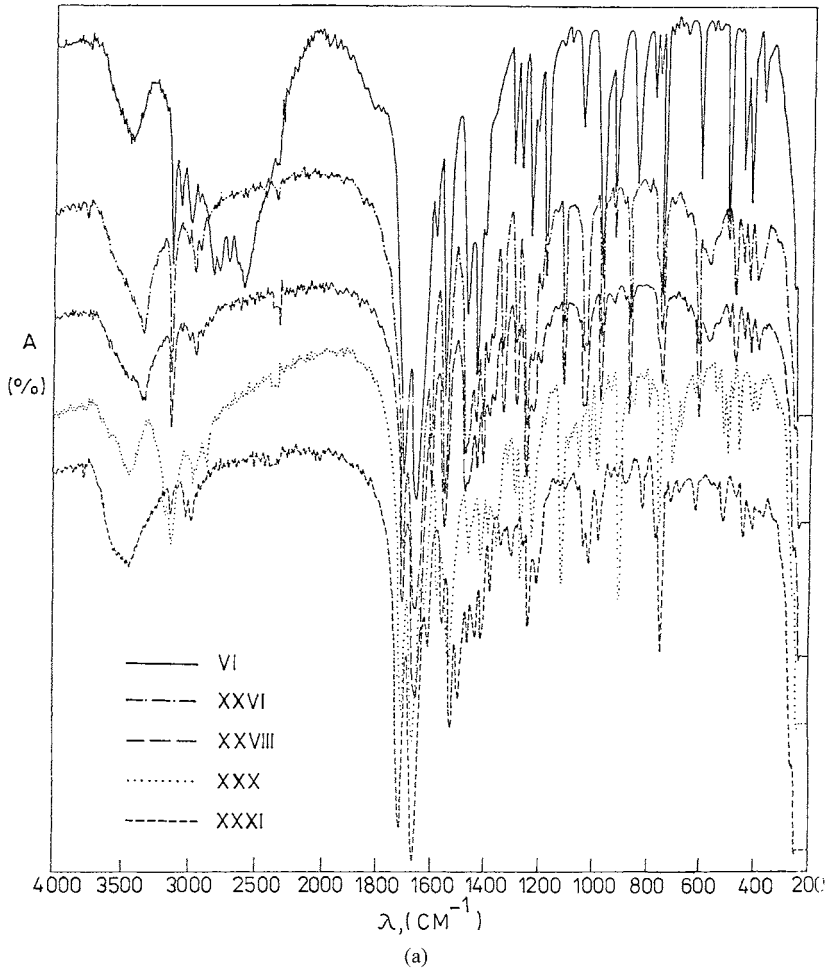


Fig. 1. IR spectra of the theophylline (**VI**) and bis-xanthine (**XXVI**) and bis-xanthine derivatives (**XXVIII**, **XXX**, and **XXXI**) (a) and **XXVII**, **XXIX**, **XXII**, and **XXXIII** (b).

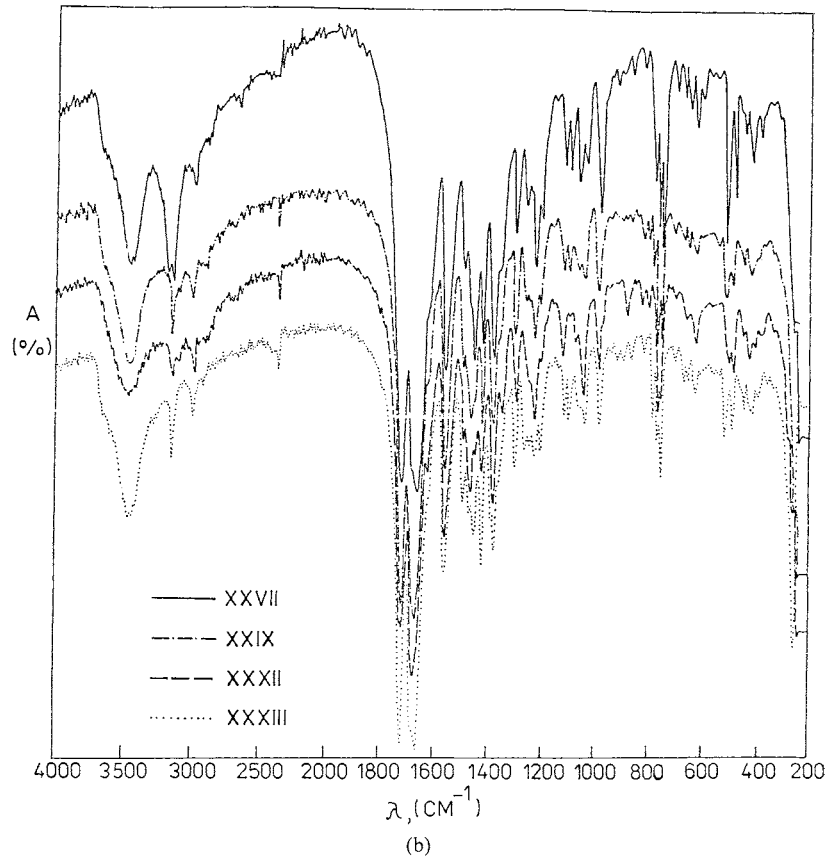


Fig. 1. (*Continued*).

mechanism of decomposition, the highest temperature of decomposition exhibiting the **XXVIII** bis-xanthine derivative, the mass loss of 77.8 wt.%, Table 6.

The onset temperature of the mass loss of the most studied compounds (except compound **XXXI**) lies above melting temperature, see the columns 2 and 4 of the Table 6, therefore the assignment of the peak of melting is correct.

Bis-xanthine derivatives with cyclic substituents decompose in two steps (I and II) in the 230–460 °C temperature interval (Fig. 3b). These bis-xanthine derivatives exhibit close thermo-oxidative behavior, therefore can suppose to be similar decomposition mechanisms.

All weight losses of bis-xanthine derivatives are smaller than those of starting materials. Generally, by inserting a substituent in the 8-position, the thermal stability of components decreases in respect to the starting compounds.

3.3. Pharmacological activity tests

The effect of the 8-R-bis-xanthine derivatives on the terminal acute inflammatory edema by ''air pouch'' method has been studied. The inflammatory edema has been induced using carrageenan. The test [15–17] has been effectuated on groups of 5 Wistar adult male rats grown, in identical laboratory conditions and having approximately the same weight of 159–160 g. The hair of the dorsal zone was removed using a spray then each rat was subcutaneous injected with 10 ml air. After 24 h from air injection and air bubble formation, a small dose of 20 mg/kg body of Evans blue was intraperitoneally delivered.

Table 2
IR spectral data for bis-xanthine derivatives

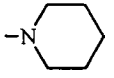
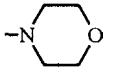
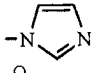
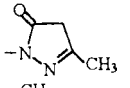
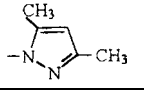
Compound	R	Frequency (cm^{-1}) of respective group											
		OH		>N–CH_3	–CH_3; –CH_2–	>C=C<$_{N-}$	>C=C<$_{CO}$	>C=N	>N-xanthine	–HC=	C–Br	C–NO_2	>N-ring
		Bound	Free										
XXVI	H	3380–3420	1310, 1200	2790	2900	1670	1600	1500	1570	980	–	–	–
XXVII	Br	3390–3400	1180	2790	2900	1680	1610	1510	1580	930	680	–	–
XXVIII	NO_2	3390	1200	2800	2900	1660	1590	1490	1570	980	–	1570	–
XXXIV		3400	1200	2790	2900	1660	1610	1480	1570	980	–	–	3120
XXIX		3400	1400	2790	2900	1670	1600	1500	1570	920	–	–	3320
XXX		3400	1310	2800	2910	1670	1610	1490	1610	930	–	–	3090
XXXI		3400	1190	2790	2900	1660	1610	1480	1570	950	–	–	3210
XXXII		3390–3410	1250	2800	2900	1670	1610	1500	1570	990	–	–	3200
XXXIII		3400	1200	2800	2910	1670	1600	1500	1570	930	–	–	3100

Table 3
^{1}H NMR spectral data for bis-xanthine derivatives

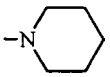
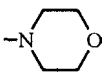
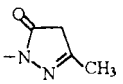
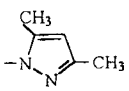
Compound	R	(δ, ppm) from ^{1}H NMR
XXVI	H	2.45 ppm (4H, 2–CH_2–N<), 3.20 ppm (6H, 2CH_3–N_1<), 3.29 ppm (6H, 2CH_3–N_3<), 3.41 ppm (1H, >CH–O–), 4.30 ppm (1H, >CH–OH), 7.28 ppm (2H, –CH=)
XXVII	Br	2.48 ppm (4H, 2–CH_2–N<), 3.20 ppm (6H, 2CH_3–N_1<), 3.29 ppm (6H, 2CH_3–N_3<), 3.60 ppm (2H, >CH–OH) 7.28 ppm (1H, –CH=)
XXVIII	NO_2	2.42 ppm (4H, 2–CH_2–N<), 3.19 ppm (6H, 2CH_3–N_1<), 3.29 ppm (6H, 2CH_3–N_3<), 3.40 ppm (1H, >CH–O–), 4.25 ppm (1H, –C–OH), 7.80 ppm (1H, –CH=)
XXIX		2.51–2.60 ppm (14H, 4–CH_2–N<, (–CH_2–)$_3$), 3.15 ppm (6H, 2CH_3–N_1<), 3.39 ppm (6H, 2CH_3–N_3<), 4.20 ppm (2H, >CH–OH), 7.80 ppm (H1, –CH=);
XXX		2.49 ppm (8H, 4–CH_2–N<), 3.21 ppm (6H, 2CH_3–N_1<), 3.32 ppm (6H, 2CH_3–N_3<), 4.30 ppm (6H, >CH–OH, 2–CH_2–O–), 7.80 ppm (1H, –CH=)
XXXII		1.82 ppm (3H, CH_3–C<), 2.48 ppm (4H, 2–CH_2–N<), 3.20 ppm (6H, 2CH_3–N_1<), 3.35 ppm (6H, 2CH_3–N_3<), 4.20 ppm (2H, >CH–OH), 7.80 ppm (1H, –CH=)
XXXIII		1.84 ppm (6H, 2CH_3–C<), 2.51 ppm (4H, 2–CH_2–N<), 3.20–3.25 ppm (6H, 2CH_3–N_1<), 3.30–3.35 ppm (6H, 2CH_3–N_3<), 4.25 ppm (2H, >C–OH), 7.88 ppm (1H, –CH=)

Table 4
Physico-chemical characteristics of bis-xanthine compounds

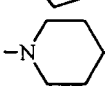
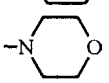
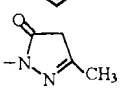
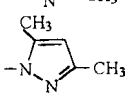
Compound	R	Molecular formula		Weight	Yield (%)	Elemental analysis (%)					
		Calculated	Theoretical			C		H		N	
						Calculated	Found	Calculated	Found	Calculated	Found
XXVI	H	$C_{17}H_{20}N_8O_5$	$C_{17}H_{20}N_8O_5$	416.41	64.90	49.03	49.21	4.84	4.98	26.91	27.08
XXVII	Br	$C_{17}H_{19}N_8O_5Br$	$C_{17}H_{19}N_8O_5Br$	495.30	60.60	41.22	41.34	3.86	3.98	22.62	22.78
XXVIII	NO_2	$C_{17}H_{19}N_9O_7$	$C_{17}H_{19}N_9O_7$	461.41	56.35	44.25	44.38	4.15	4.31	27.32	27.45
XXXIV		$C_{21}H_{27}N_9O_5$	$C_{21}H_{27}N_9O_5$	485.52	47.93	51.95	52.08	5.60	5.74	25.96	26.14
XXIX		$C_{22}H_{29}N_9O_5$	$C_{22}H_{29}N_9O_5$	499.51	58.11	52.90	53.04	5.85	5.98	25.24	25.36
XXX		$C_{22}H_{29}N_9O_5$	$C_{22}H_{29}N_9O_5$	501.52	47.90	50.21	50.34	5.42	5.56	25.13	25.28
XXXI		$C_{22}H_{29}N_9O_5$	$C_{22}H_{29}N_9O_5$	482.48	60.16	49.78	49.96	4.59	4.76	29.03	29.21
XXXII		$C_{21}H_{24}N_{10}O_6$	$C_{21}H_{24}N_{10}O_6$	512.50	64.45	49.21	49.38	4.72	4.48	27.33	27.48
XXXIII		$C_{22}H_{26}N_{10}O_5$	$C_{22}H_{26}N_{10}O_5$	510.55	78.35	51.57	51.74	5.13	5.25	27.43	27.61

Table 5
The solubility of bis-xanthine derivatives in polar and non-polar solvents[a]

Solvent	Compound								
	XXVI	**XXVII**	**XXVIII**	**XXXIV**	**XXIX**	**XXX**	**XXXI**	**XXXII**	**XXXIII**
Water	+	+	++	−	+	+	−	+	+
Methanol	+	−	+	−	+	+	−	+	+
Ethanol	+	+	+	−	+	+	−	+	+
Propylalcohol	−	++	+	+	+	+	−	++	+
Isopropyl alcohol	−	−	−	+	+	−	−	+	+
Acetone	−	−	−	−	+	−	−	−	+
Chloroform	−	−	−	−	+	+	−	++	+
Dioxane	−	+	−	−	+	+	−	++	+
Benzene	+	−	−	−	−	−	−	−	−
Ethyl ether	−	−	−	−	−	−	−	−	−
DMF	++	++	++	++	++	++	+	++	++
DMSO	++	++	++	++	++	++	++	++	++

[a] (−) insoluble; (+) partial soluble; (++) soluble in hot solvent.

Each set of five rats received in the next step, by an orally gavage, a xanthine derivative in form of a suspension in a sodium carboxymethyl cellulose solution of 0.5 wt.% concentration, which was diluted with 1 ml saline solution (serum). The delivered dose was of 25 mg/kg body and was performed immediately after Evans blue delivery. All tests were compared with a reference one consisting in a set of 5 Wistar adult male rats that, has been only 1 ml serum, delivered by orally gavage. All sets of rats including reference set have been finally subcutaneously injected directly in air bubbles formed with 24 h before, with 6 ml carrageenan solution 2/1 (v/v) concentration in serum.

After 24 h, the rats were killed and air bubble was opened. The tissue and subcutaneously material of dark blue color were removed, retaining 100 mg from each sacrificed animal.

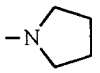
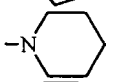
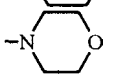
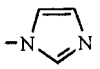
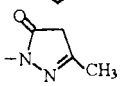
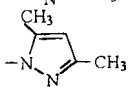
This biological material was dispersed in 5 ml serum and 5 ml mixture of 7/3 (v/v) acetone containing 5 g/l sodium sulfate in order to extract the Evans blue from tissue.

The samples were stored 24 h at room temperature, and then were centrifuged. The sediment was rejected

Table 6
DSC and TG/DTG data for bis-xanthine derivatives

Compound code	DSC data		TG/DTG data			
	T_m (°C)	ΔH (kJ/mol)	T_1 (°C)	T_m (°C)	T_f (°C)	Δw (%)
VI	274.3	23.7	226	384	434	85.6
XXVI	270	28.9	275	446	497	80.1
XXVII	224	28.4	224	278.5	311	19.9
XXVII	–	–	311	366	492.5	47.6
XXVIII	275.3	50.8	300	444	494.5	77.8
XXIX	219	33.7	232	268.5	316	17.3
XXIX	–	–	316	364.5	450	46.5
XXX	227	11.3	252	371	476	64
XXXI	285	–	252	358	450.5	45.9
XXXII	–	–	239	282	301	20
XXXII	–	–	–	363	430	42.3
XXXIII	231.5	39.3	241	291	319	20.5
XXXIII	–	–	319	370	443	45.2

Table 7
Effect of bis-xanthine derivatives on inflammatory edema induced by carrageenan

Compound	R	Delivered quantity (mg/kg)	Evans blue concentrations (μg/ml)	Retained inflammation (%)
XXVI	H	27.00	9.95 ± 1.82[b]	34.68[b]
XXVII	Br	32.19	11.73 ± 2.3[b]	40.88[b]
XXVIII	NO_2	27.00	20.65 ± 3.49[a]	71.97[a]
XXXIV		31.55	5.92 ± 1.97[b]	20.63[b]
XXIX		32.46	7.30 ± 1.50[b]	25.44[b]
XXX		32.59	6.84 ± 1.43[b]	23.84[b]
XXXI		31.36	4.91 ± 1.04[b]	17.11[b]
XXXII		33.31	18.55 ± 2.37[a]	64.65[a]
XXXIII		33.18	13.58 ± 2.90[a]	47.33[a]
VI	Theophylline	30.00	17.53 ± 4.16[a]	61.10[a]
M	Reference set (treated with carrageenan)	–	28.69 ± 5.60	100

[a] Statistically significant values ($P < 0.05$) in respect with the reference set.
[b] Statistically significant values ($P < 0.05$) in respect with theophylline.

and the supernatant was used for quantitative determination of the Evans blue. The Evans blue quantity was spectrophotometrically determined by means of a Hewlett-Packard HP 8453 UV–VIS spectrophotometer provided with a HP-Chemstation software. The characteristic wavelength of UV spectra recorded was 629 nm. The calibration curve was plotted with solutions of known concentration of Evans blue ranging from 1 to 200 μM in mixture of serum/acetone/sodium sulfate 5 g/l.

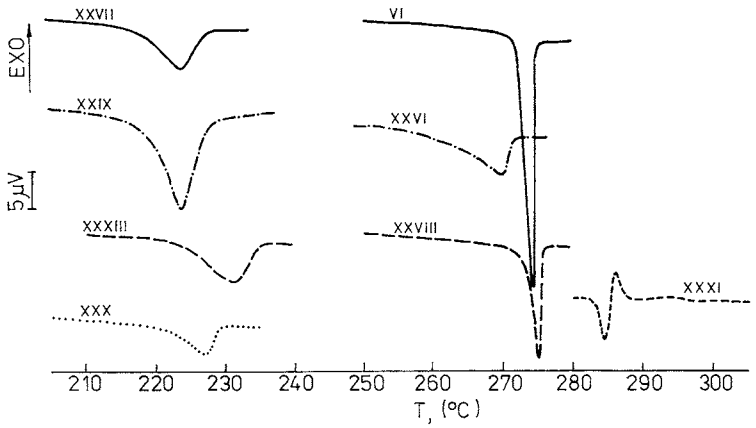


Fig. 2. DSC curves of the theophylline (**VI**) and bis-xanthine (**XXVI**) and bis-xanthine derivatives.

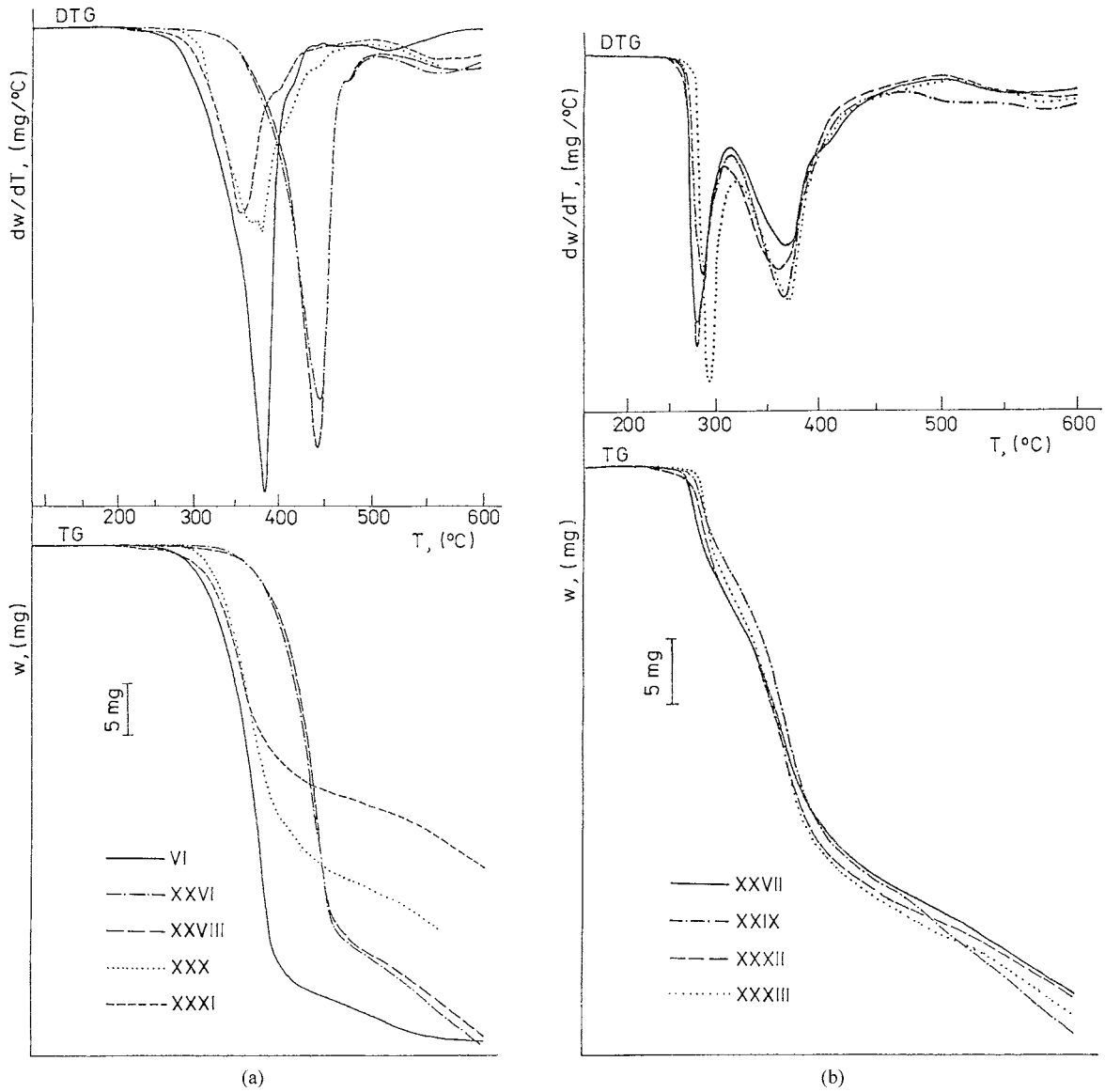


Fig. 3. TG/DTG curves of the theophylline (**VI**) and bis-xanthine (**XXVI**) and bis-xanthine derivatives (**XXVIII**, **XXX** and **XXXI**) (a) and **XXVII**, **XXIX**, **XXII**, and **XXXIII** (b).

Statistic interpretation of data was made by one-way analysis of variance (ANOVA) test corrected with ANOVA on ranks (Student–Newman–Keuls method) or with Bonferoni test; the last consisting in comparative examination of each test with the reference one. The probability error (P) values smaller than 0.05 in respect to reference set or to theophylline have been considered statistically significant.

The effect on the inflammatory acute edema was counted, by the concentration of Evans blue (Evans blue μg/ml) and retained inflammation as percent of inflammation produced by tested compound in respect to reference test (the retained inflammation of the reference was considered 100%).

The most significant anti-inflammatory effect is appreciated when these two characteristics exhibit

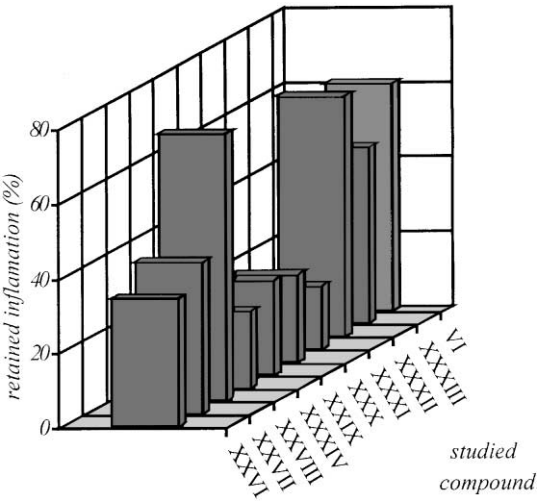


Fig. 4. Effect of bis-xanthine derivatives on inflammatory edema induced by carrageenan.

minimum values. The results are presented in the Table 7 and Fig. 4. The bis-xanthine derivatives have similar bronchodilatator activity with the theophylline but in the same time have superior anti-inflammatory activity comparing with theophylline. The most efficient are compounds **XXXIV** and **XXXI**.

4. Conclusions

The new synthesized bis-xanthine derivatives exhibit high melting points and decompositions temperatures. Therefore, they are stable during thermal treatments used for their processing. By coupling DSC and TG results, the endothermic peak of DSC curves were correctly assigned. The thermal behavior is changed depending on the chemical nature of the substituents.

Both DSC and TG curves could be used for the bis-xanthine derivatives identification. This is very useful information, taking in view their selective activity on bronchodilatator and/or cardiovascular systems.

References

[1] S. Corsano, R. Scapichi, G. Strapaghetti, R. Coulson, S.J. Scheiman, Therapeutics 248 (2) (1988) 589–595; Chem. Abstr. 122 (1996) 9766z.
[2] G.E. d'Alonso, Am. Rev. Respir. Dis. 147 (1990) 165–184.
[3] P. Donoso, S.C. O'Neill, K.W. Dilly, N. Nigretti, D.A. Eisner, Br. J. Pharmacol. 111 (2) (1994) 455–458.
[4] J.P. Finnerty, C. Lee, S. Wilson, J. Madden, R. Djukanovic, S.T. Holgate, Eur. Respir. J. 9 (8) (1996) 1672–1677.
[5] V.E. Whitwhurst, X. Joseph, T.A. Vick, F.R. Alleva, J. Zhang, T. Balazs, Toxicology 110 (1–3) (1996) 113–121.
[6] Gh. Danila, L. Profire, G.G. Bumbu, C. Vasile, Thermochim. Acta 343 (2000) 69–79.
[7] L. Profire, M. Costuleanu, G.G. Bumbu, C. Vasile, in: Proceedings of the XV-eme Session des Journées Médicales Balkaniques, 28–30 April 1999, pp. 166–172.
[8] L. Profire, Studies on the Obtaining by Semi-Synthesis of Some New Bioactive Xanthine Derivatives, PhD Thesis, Gr. T. Popa Medicine and Pharmacy University of Jassy, July 1999.
[9] A. Zejc, M. Gorczyca, K. Kieckononowicz, M. Pawlowski, Pol. J. Pharmacol. Pharm. 20 (1963) 304.
[10] J.L. Ford, P. Timmins, Pharmaceutical Thermal Analysis; Techniques and Applications, Ellis Horwood, Chichester, 1989.
[11] J. Haleblian, W. McCrone, J. Pharm. Sci. 58 (8) (1969) 911.
[12] G.P. Bettinetti, Il Farmaco Ed. Pr. 48 (3) (1988) 71.
[13] J.I. Wells, Pharmaceutical Preformulation, Wiley, 1988.
[14] Operating Instructions, Mettler, Switzerland, 1990.
[15] J.R. Dryer, Applications of Absorption Spectroscopy of Organic Compounds, Prentice-Hall, Englewood, 1965, p. 22.
[16] J.L. Black, C.L. Armour, P.R.A. Johnson, L.A. Alouan, P.J. Barnes, Am. Rev. Respir. Dis. 142 (1990) 1384.
[17] K.H. Banner, C.P. Page, Eur. Respir. J. 8 (1995) 996.