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Short communication

Synthesis and anticonvulsive activity of thiolosigamone

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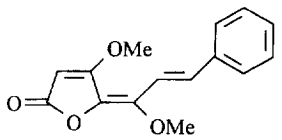
Abstract – By diastereoselective aldol-type reaction of 2-chlorobenzaldehyde with methyl thiotetronate **4**, racemic thiolactone **3a**, an analogue of losigamone **2**, was synthesized along with its (*R**,*R**)-isomer **3b** and its dehydration product **5**. In contrast to the lead compound, thiolosigamone **3a** displayed weak anticonvulsant activity only at high doses in the MES-test. The other newly synthesized compounds did not protect mice against seizures induced by maximal electroshock. © Elsevier, Paris

losigamone / aldol reaction / bioisosterism / anticonvulsant

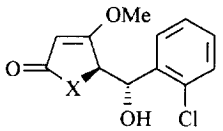
1. Introduction

In 1971 Hänsel and Pelter [1] reported on the isolation of a cinnamylidene butenolide from Mexican *piper sanctum* (Miq.) Schlecht which they named piperolide **1** (*figure 1*). The chemical structure of piperolide was confirmed by synthesis [2-5] and later by X-ray diffraction analysis [6]. Results of some early pharmacological studies indicated that the isolated tetronic acid derivative piperolide possesses depressant activities on the central nervous system of experimental animals [7, 8]. Such reports stimulated systematic structure-activity studies (SAR) of tetronic acid derivatives [9, 10] which eventually led to the identification of losigamone **2** as a potential antiepileptic agent suitable for further development [11]. This novel tetronate is now undergoing extensive clinical trials and its unique pharmacological activity profile and current state of development have also been reviewed [12a].

In the systematic structure-activity studies leading to the selection of losigamone, no due considerations were given to the importance of the tetronic acid part of the molecule. Now attempts are being made to clarify the situation and to test whether modulation of the five-membered ring system could eventually lead to better anticonvulsants with prolonged durations of action. The present communication deals with efforts in which the ring oxygen atom of the lactone moiety was replaced by sulphur. For brevity's sake the (*R**,*S**)-configured thiolactone **3a** (*figure 1*), an analogue of losigamone **2**, will be refered to as thiolosigamone.



Piperolide **1**



X = O: Losigamone **2** *(rac.)*
X = S: Thiolosigamone **3a** *(rac.)*

Figure 1.

It is known that by exchange of a lactone versus the thiolactone moiety the isosteric compounds in some cases exhibit similar or modified physiological activities, as exemplified by thiopilocarpin [13], thiobasidalin [14] or thioascorbic acid [15]. Hence the use of the term 'bio-

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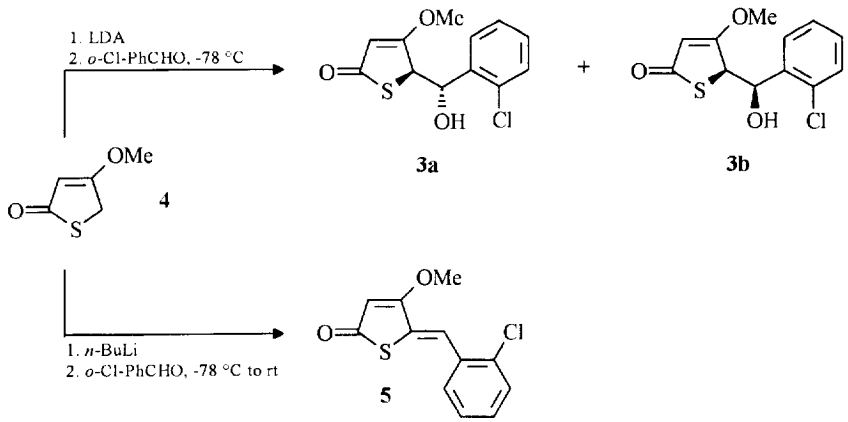

Figure 2.

isosterism' [16] might be appropriate in those cases as well as in the cases examined here.

2. Chemistry

Based upon earlier studies with thiolactones [14, 15], *o*-chlorobenzaldehyde was directly added to lithiated thiotetronic acid ether **4** [17]. After work-up at low temperature we obtained a mixture of diastereomers **3a/3b** in a ratio of 85:15 as evidenced by HPLC analysis of the crude reaction mixture (*figure 2*). The isomers were easily separated by flash chromatography.

The structure of the predominant aldol was unambiguously clarified by X-ray diffraction analysis (*figure 3*) as the desired *threo*-configured racemic 1-thialosigamone analogue **3a**.

If the work-up of the metallated aldol reaction mixture containing **3a** and **3b** was conducted at room temperature, dehydration took place to furnish the (*Z*)-benzylidene thiotetronate **5** in good overall yield. Only one geometrical isomer could be detected. The positive NOE of the exocyclic vinylic hydrogen generated by irradiation on the methoxy signal proved the (*Z*)-configuration.

The partition coefficient of **3a** between *n*-octanol and water was determined to 74.2:1, equivalent to a log *P* of 1.87; in comparison, the log *P* of losigamone **2** is 1.52.

3. Pharmacology

Potential anticonvulsant activities of the synthesized thiolactones in mice maximal electroshock model (MES-model) were compared with that of their lead substance losigamone **2**. Choice of this model for such purposes was based upon the known and well-defined pharmacological activity profile of losigamone [10-12] and on our current understanding of the structure-activity relationship of losigamone analogues and derivatives in this model [9]. It must be pointed out that the anticonvulsant activity profile of losigamone in rats and mice, as judged by its ED_{50} in various seizure models, is not comparable to any other known antiepileptic drug or agent currently under development [12]. In addition, except in the MES-model, the efficacy of losigamone in other seizure models

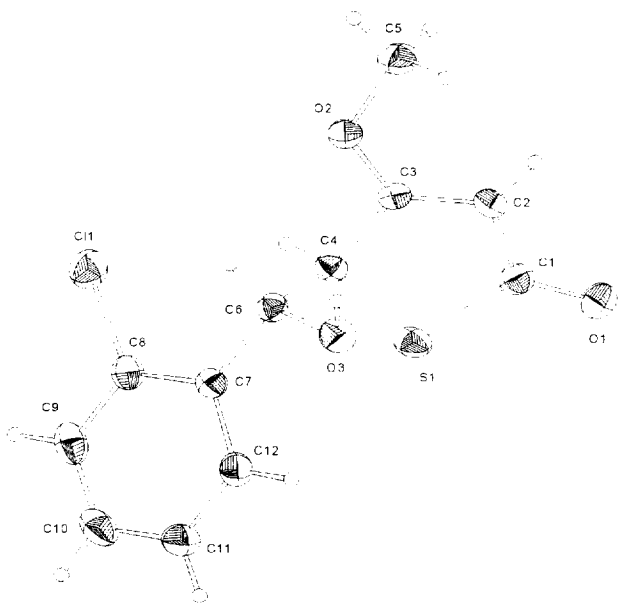

Figure 3. ORTEP-plot of thiolosigamone **3a**.

Table I. Anticonvulsive activity of thiolactones and losigamone in mice MES-test.

Substance	Oral dose (mg/kg)	Number of animals/group	% protection against extensor seizures: time after oral application	
			1 h	3 h
4	250[a]	8	0	0
5	250	8	0	0
3a	100	8	0	0
	250[a]	8	40	0
3b	250	8	0	0
2	20	8	100	0
	40	10	100	80

[a] Sedation, ataxia and other respiratory depressions were observed in all animals and such effects of the agents persisted in some even 3 h after administration.

depends not only on the species but also on the animal strain used.

In an initial series of experiments no seizure protecting activities of the thiolactone derivatives described could be detected in animals treated with 100 mg/kg (p.o.) doses of the agents and tested one or three hours thereafter (n = 8/group) as depicted in the table below. As expected, however, 20 mg/kg dose of losigamone **2** protected all animals from seizures only one hour after oral administration and was found to be inactive when tested three hours after the administration (n = 8).

In a second series of experiments higher doses were tested. It can be seen that, although thiolosigamone **3a** displays some anti-seizure activity, its duration of action is short and that 250 mg/kg dosages necessary to observe anticonvulsant activity of the agent were at least equal (if not higher) to its sedative or ataxic doses. On the contrary, only 40 mg/kg dose of losigamone **2** afforded 80% protection even after three hours after oral administration and no behavioural disturbances were observable after such doses. Earlier data from our laboratories and elsewhere [9, 11] have repeatedly demonstrated that the 1 h oral ED_{50} of losigamone in mice MES test is below 20 mg/kg and that even 100 mg/kg doses of the agent do not induce sedation or ataxia in mice. The behavioural changes observed after 250 mg/kg doses of thiolosigamone **3a** in this study were, however, qualitatively very similar to those observed after similar oral doses of losigamone **2**. Hence it appears certain that the therapeutic index of thiolosigamone is close to or even lower than 1 whereas that of losigamone is much higher than 5.

In view of these findings the absence of anticonvulsant activity in the other three newly synthesized thiolactones **3b**, **4** and **5** is not surprising. Our earlier structure-activity studies have demonstrated that the corresponding oxa-analogues of **4** and **5** are inactive as anticonvulsants and the *erythro*-isomer of losigamone (i.e. the oxa-analogue of **3b**) is by far less potent than losigamone itself [12a]. It must, however, be pointed out that the *erythro*-isomer of losigamone **2** afforded 100% protection after an oral dose of 250 mg/kg [12a] whereas after similar dosages the corresponding sulphur analogue **3b** did not exert any anticonvulsant activity.

4. Conclusion

Bioisosteric displacement of the ring oxygen atom of losigamone **2** by sulphur forming thiolosigamone **3a** drastically reduces its therapeutic index as an anticonvulsant. This replacement specifically affects the anticonvulsant efficacy of the molecule but not its side effect potentials.

5. Experimental protocols

5.1. General methods

Melting points were determined using a Gallenkamp Melting Point apparatus and are uncorrected. Flash chromatography was usually performed using silica gel (230-400 mesh) from Merck. ^1H and ^{13}C NMR spectra were recorded using either Me_4Si or the solvent peak as internal standard on a JEOL GSX 400 and on a Bruker AC 200 spectrometer. Mass spectra were obtained with a Hewlett Packard 5989A Mass Spectrometer employing both EI (70 eV) and CI mode or a VG Trio-2000 (Micromass) Mass Spectrometer with electrospray ionization (ES+, 42 eV). Infrared spectra were measured as KBr plates using a FT-IR-Spectrometer PARAGON 1000 or IR Spectrometer 197 (Perkin-Elmer). UV analysis was performed in methanolic solutions on Uvikon 810 Anakomp 220 (Kontron), UV/VIS Spectrometer Lambda 14 and 20, respectively (Perkin Elmer). HPLC analysis was made employing Merck-Hitachi L-6000A/L-4000A and LiChrospher® 100 DIOL, 10 µm (Merck) eluting with hexane/ethyl acetate mixtures. THF was distilled from sodium benzophenone ketyl under

N_2 immediately prior to use. All moisture-sensitive reactions were run with flame-dried glassware.

5.2. Maximal electroshock test (MES-test)

These tests were carried out on male albino mice (NMRI/Charles River LF no. 1) using the standardized procedure described in an earlier publication [12d] dealing with the isomers of losigamone.

*5.3. 5-[(2-Chlorophenyl)hydroxymethyl]-4-methoxy-2(5H)-thiophenone **3a/3b***

To a dry ice-cooled solution of diisopropylamine (2.3 mL, 17.5 mmol) in THF (100 mL) was dropwise added under N_2 *n*-butyllithium (1.6 M, 10 mL, 16 mmol) and the solution was stirred for 30 min at this temperature. Thereupon a solution of 4-methoxy-2(5H)thiophenone **4** (2.02 g, 15.5 mmol) in THF (30 mL) was slowly added dropwise. After another hour at – 78 °C a precooled (– 78 °C) solution of 2-chlorobenzaldehyde (2.25 g, 16 mmol) in THF (10 mL) was added all at once. The mixture was stirred for another 15 min and then hydrolyzed by addition of sat aq citric acid solution. The mixture was extracted with ethyl acetate (3 × 30 mL) and the combined dried (Na_2SO_4) organic extracts were evaporated. The remaining residue was subjected to flash chromatography using chloroform/diethyl ether (6:1) as eluent. The more mobile spot (TLC), the *erythro*-isomer **3b** was obtained first. 569 mg (13.5%), R_f 0.25. Thereupon the main product, the *threo*-isomer **3a** was eluted. 3.218 g (76.5%). R_f 0.12. Diastereomeric ratio (**3a/3b**): 85:15 (HPLC).

5.4. (R,S*)-5-[(2-Chlorophenyl)hydroxymethyl]-4-methoxy-2(5H)-thiophenone, thiolosigamone **3a***

Colourless crystals, m.p. 128 °C (diisopropyl ether/ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 7.56 (m, 1 H, ar H), 7.30-7.17 (m, 3 H, ar H), 5.45 (dd, 1 H, J_1 = 4.7 Hz, J_2 = 3.0 Hz; after exchange with D_2O: d, 1 H, J = 4.7 Hz), 5.35 (s, 1 H), 4.76 (d, 1 H, J = 4.7 Hz), 3.68 (s, 3 H), 3.01 (d, 1 H, J = 3.0 Hz, OH); ^{1}H NMR ([d_6]-DMSO) δ 7.66-7.61 (m, 1 H, ar H), 7.45-7.28 (m, 3H, ar H), 6.05 (dd, 1 H, $J_{OH,\alpha}$ = 5.2 Hz, $J_{OH,5}$ = 0.5 Hz, OH), 5.74 (d, 1 H, $J_{3,5}$ = 0.8 Hz, H-3), 5.55 (dd, 1 H, $J_{OH,\alpha}$ = 5.2 Hz, $J_{\alpha,5}$ = 2.8 Hz, H-α, 4.75 (d, 1 H, $J_{\alpha,5}$ = 2.8 Hz, H-5), 3.90 (s, 3 H, OCH_3); ^{13}C-NMR ([d_6]-DMSO) (193.7 (s, C-2), 183.2 (s, C-4), 139.9 (s, C-1'), 130.3 (s, C-2'), 129.3, 128.9, 128.4, 127.1 (all d, C-3'-C-6'), 103.7 (d, C-3), 66.6 (d, C-α, 60.1 (q, OCH_3), 55.9 (d, C-5); IR ν 3345, 1655, 1589, 1440 cm^{-1}; UV λ_{max}(lg ε) 217 nm (4.166), 234 (4.177), 260 sh (3.689); Anal. $C_{12}H_{11}ClO_3S$ (270.74) Calcd C, 53.24; H, 4.10; Cl, 13.10; S, 11.84; Found C, 53.24; H, 4.15; Cl, 13.00; S, 12.21%. MS: 271/273, ratio: 3:1 [M^+].

5.5. X-ray diffraction analysis

A suitable crystal (size 0.27 × 0.40 × 0.47 mm) of octahedral shape was obtained by slow evaporation of an ethyl acetate solution: $C_{12}H_{11}ClO_3S$, M = 270.74, monoclinic, Space group $P2_1/c$, a = 10.835(1), b = 9.022(1), c = 13.321(3) Å, β = 108.445(12)°, V = 1235.2(3) Å^3, Z = 4, D_{calc} = 1.456 Mg.m^{-3}, λ Mo-K_α) = 0.71073 Å, F(000) = 560, μ = 0.470 mm^{-1}. Cell parameters were obtained by least squares refinement of 25 reflections in the range of 9°< Θ < 120°. The data collection was performed at room temperature using graphite monochromized Mo K_α radiation on a Nonius CAD4 diffractometer; ω-2Θ-scans, scan width [0.78 + 0.43 tan Θ]° and a maximum measuring time of 45 s up to a Θ_{max} value of 23°. Three standard reflections were measured every 2 h and showed an intensity decay of 2.1%. The corrections for *Lp*, linear decay and absorption (T_{min} = 0.9055, T_{max} = 0.9991) were applied on a total of 1796 reflections, 1710 unique and 1478 with $I > 2\sigma I$. All non-hydrogen atoms were refined anisotropically. The final R1 was 0.0269 ($wR2$ = 0.0683) for 1478 reflections and 157 variables and 0 restraint and R1 = 0.0336 ($wR2$ = 0.0734) for all 1710 data. The structure was solved using SHELXS-86 [18] and refined by SHELXL-93 [19] against F^2. Weights: SHELXL-93, Extinction coefficient 0.0126(13). The final residual electron density was 0.188 and – 0.166 eÅ^{-3}. The drawing was made using XPMA, ZORTEP [20]. The calculations were performed on a Pentium-PC. The structure is characterized by an intermolecular hydrogen bridge from the alcoholic hydrogen to the carbonyl oxygen with a distance of 1.943 Å.

Complete details of the structure investigation are available on request from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, England on quoting the names of the authors and the journal citation.

5.6. (R,R*)-5-[(2-Chlorophenyl)hydroxymethyl]-4-methoxy-2(5H)-thiophenone **3b***

Colourless crystals, m.p. 141 °C (diisopropyl ether/ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 7.45-7.40 (m, 1 H, ar H), 7.29-7.17 (m, 3H, ar H), 5.69 (dd, 1 H, J_1 = 2.6 Hz, J_2 = 8.6 Hz; after exchange with D_2O: d, 1 H, J = 8.6 Hz), 5.45 (s, 1 H), 4.77 (d, 1 H, J = 2.6 Hz), 3.87 (s, 3 H, OCH_3), 2.83 (d, 1 H, J = 8.6 Hz, OH); ^{1}H NMR ([d_6]-DMSO) (7.71-7.66 (m, 1 H, ar H), 7.41-7.27 (m, 3H, ar H), 6.25 (d, 1 H, $J_{OH,\alpha}$ = 4.7 Hz, OH), 5.54 (d, 1 H, $J_{3,5}$ = 0.5 Hz, H-3), 5.50 (t, 1 H, $J_{OH,\alpha} \cong J_{\alpha,5}$ = 4.3 Hz, H-α, 4.90 (dd, 1 H, $J_{\alpha,5}$ = 4.0 Hz, $J_{3,5}$ = 0.5 Hz, H-5), 3.72 (s, 3 H); ^{13}C-NMR NMR ([d_6]-DMSO) δ 193.2 (s, C-2), 183.2 (s, C-4), 138.1 (s, C-1'), 131.1 (s, C-2'), 130.8, 129.2, 128.7, 126.3 (all d, C-3'-C-6'), 103.1 (d, C-3), 68.9 (d, C-α), 59.6 (q, OCH_3), 55.8 (d, C-5); IR (3341, 1653, 1604, 1439 cm^{-1}; UV λ_{max}(lg ε) 233 nm (4.072), 260 sh (3.624); Anal. $C_{12}H_{11}ClO_3S$ (270.74) Calcd C, 53.24; H, 4.10; Cl, 13.10; S, 11.84; Found C, 53.21; H, 4.13; Cl, 12.97; S, 12.00%. MS: 271/273, ratio: 3:1 [M^+].

*5.7. (Z)-5-(2-Chlorophenylmethylene)-4-methoxy-2(5H)-thiophenone **5***

To a dry ice-cooled solution of *n*-butyllithium (1.6 M, 26.3 mL, 42 mmol) in THF (150 mL) was dropwise added under N_2 a solution of 4-methoxy-2(5H)thiophenone **4** (2.02 g, 15.5 mmol) in THF (150 mL). The resulting suspension was stirred at – 35 °C for 30 min, thereupon recooled to – 78 °C and charged with 2-chlorobenzaldehyde (26.7 g, 190 mmol). The mixture was allowed to warm to 10 °C overnight and was then acidified by addition of dil hydrochloric acid. The volatiles were removed in vacuo and the resulting residue was taken up with chloroform (300 mL), washed with water, dried (Na_2SO_4) and evaporated to dryness. Flash chromatography of the remainder using chloroform as eluent gave **5** (7.0 g, 74%). Colourless crystals, m.p. 125-126 °C (isopropanol). ^{1}H NMR ([d_6]-DMSO) δ 7.66-7.58 (m, 3 H, H_α and

ar H), 7.52-7.40 (m, 2 H, ar H), 6.08 (s, 1 H, H-3), 4.04 (s, 3 H); ^{13}C-NMR NMR ([d_6]-DMSO) δ 188.5 (s, C-2), 175.2 (s, C-4), 134.0, 132.1, 130.8 (all s, C-1', C-2', C-5), 131.1, 130.0, 129.6, 127.8 (all d, C-3'-C-6'), 122.9 (d, C-α), 102.2 (d, C-3), 60.3 (q, OCH_3); IR (3080, 1685, 1585, 1435 cm^{-1}; UV λ_{max}(lg ε) 206 nm (4.450), 240 (3.911), 248 sh (3.812), 325 (4.283); Anal. $C_{12}H_9ClO_2S$ (252.74); Calc. C, 57.03; H, 3.59; Cl, 14.03; S, 12.69; Found C, 56.94; H, 3.60; Cl, 14.9, S, 12.8%. MS: 253/255, ratio: 3:1 [M^+].

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