Antispasmodic Activity of Xanthoxyline Derivatives: Structure–Activity Relationships

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Abstract □ The antispasmodic activity of several xanthoxyline derivatives against acetylcholine-induced contraction of the guinea pig ileum was evaluated in vitro. The acetophenones with two methoxyl groups, mainly in the 3,4 positions, exhibited potent antispasmodic activity. Modification of the hydroxyl group in xanthoxyline by the introduction of benzoyl, acetyl, or tosyl groups produced inactive compounds, whereas the introduction of benzyl or *p*-methoxybenzyl groups furnished compounds that were four- to eight-fold more potent than xanthoxyline. In marked contrast, the introduction of a methyl group gave a compound that caused contractant activity. Modification of the carbonyl group of xanthoxyline lead to inactive compounds, whereas the condensation of xanthoxyline with benzaldehydes gave chalkones that were about fivefold more potent than xanthoxyline. The introduction of benzyl and styrene groups, on the basis of the similarity with papaverine, improves the antispasmodic action of the xanthoxyline derivatives. Our results suggest that the methoxyl and carbonyl groups are critical structural points for the antispasmodic activity of xanthoxyline derivatives. The hydroxyl group improves antispasmodic activity, but is not fundamental to its manifestation.

2-Hydroxy-4,6-dimethoxyacetophenone (xanthoxyline; **1**) is the major constituent isolated from the hexane extracts of *Sebastiania schottiana* (Euphorbiaceae), a native Brazilian medicinal plant used in folk medicine for the treatment of kidney disease. Xanthoxyline has also been isolated from other plants, such as *Fagara okinawaensis* (Rutaceae),[1] *Pulicaria undulata* (Compositae),[2] *Eucalyptus michaeliana* (Myrtaceae),[3] *Sapium sebiferum* (Euphorbiaceae),[4] and *Phyllanthus sellowianus* (Euphorbiaceae).[5] We have previously demonstrated that the extract of *Sebastiania schottiana* and xanthoxyline caused a potent and concentration-dependent noncompetitive antagonistic effect against several agonist-mediated contractions of the ileum and urinary bladder from guinea pig and rat uterine smooth muscles in vitro, with 50% inhibitory concentration with IC_{50} values ranging from 47 to 190 μM. In addition, xanthoxyline also inhibited, in a graded manner, twitch responses evoked by electrical stimulation of strips of guinea pig longitudinal ileum, urinary bladder, dog ureter, and rat left atrium, with IC_{50} values of 50 to 480 μM.[6–8] We have also recently reported that xanthoxyline exhibited antibacterial activity against some bacteria frequently found in the urinary tract.[9]

The present study was therefore designed to produce several xanthoxyline derivatives by structural modification and to analyze their structure–activity relationships, determining the structural groups responsible for their in vitro antispasmodic activity against acetylcholine-induced contraction of the guinea pig ileum to achieve more active compounds.

Experimental Section

Chemical Procedures—Melting points were determined with a Microquimica AP-300 apparatus and were uncorrected. Elementary analyses were obtained on a Perkin Elmer 2400. Percentages of C, H, N were in agreement with the product formula. IR spectra were recorded with a Perkin-Elmer 720 spectrometer on KBr disks or in a liquid film on NaCl disks. The ^{1}H-NMR spectra were recorded on a Varian XL 60 or 100 MHz and on a Bruker 270 or 200 MHz. Compounds were dissolved in deuterated solvents from commercial sources with tetramethylsilane (TMS) as the internal standard. The purity of the synthesized substances was monitored by thin-layer chromatography (TLC) with 200-μm thick silica pre-coated plastic plates (Sigma), with several solvent systems of different polarity. Some compounds were purified by preparative TLC (PTLC) performed with Kiesegel 60 PF254 Merck plates (1.0–2.0 mm in thickness) that were activated at 105 °C for 1–2 h prior to use. Spots were visualized by shortwave UV light and iodine vapor. The solvents and reagents were purified in the usual manner. Compounds **2**–**4** were purchased from commercial sources. 3,4-Dimethoxyacetophenone (**5**) was donated by Prof. Franco Delle Monache, Universita Cattolica S. Cuore, Rome, Italy.

Procedure for Isolation of Xanthoxyline—Dried stems and leaves of *Sebastiania schottiana* were extracted with 90% MeOH at room temperature. The extract was concentrated, diluted with water, and treated with hexane. The hexane extract was chromatographed on a Si-gel (toluene) column to give xanthoxyline (**1**; yield, 0.25%); mp 81 °C; IR (KBr): 3460, 2925, 1615, 1460, and 1385 cm^{-1}; ^{1}H NMR (100 MHz, $CDCl_3$): 2.60 (s, 3H, CH_3); 2.82 (s, 3H, CH_3O); 3.85 (s, 3H, CH_3O); 5.92 (d, J = 2.5 Hz, 1H); 6.06 (d, J = 2.5 Hz, 1H, 2 arom H); and 14.03 ppm (s, 1H, OH).

Synthesis of Xanthoxyline Derivatives—*2,4,6-Trimethoxyacetophenone* (***6***)—Xanthoxyline (0.14 g) was dissolved in acetone (3 mL), $(CH_3)_2SO_4$ (0.106 g), and 10% NaOH (1 mL). The mixture was allowed to stand for several hours, the solvent was evaporated, and the crude product was purified by PTLC (hexane:ethyl acetate, 2:1) to give **6** (yield, 0.083 g or 55%); mp, 103 °C (Lit:[1] 104 °C); IR (KBr): 2900, 1680, 1600, and 1370 cm^{-1}; ^{1}H-NMR (60 MHz, $CDCl_3$): 2.20 (s, 3H, CH_3); 3.70 (s, 3H, CH_3O); 3.82 (s, 3H, CH_3O); 3.86 (s, 3H, CH_3O); and 6.10 ppm (2d, 2 arom. H).

Oxime Derivative (***8***)—Xanthoxyline (0.2 g) was dissolved in a mixture containing hydroxylamine hydrochloride (0.5 g) and 10% NaOH (3 mL). The solution was refluxed for 3 h and poured into cool water, and the solid was recrystallized from benzene to give **8** (yield, 0.145 g or 73%); mp, 106 °C (lit:[4] 108–109 °C); IR (KBr): 3250, 1640, 1600, and 1375 cm^{-1}; ^{1}H-NMR (60 MHz, $CDCl_3$): 2.25 (s, 3H, CH_3); 3.70 (ds, 6H, 2-CH_3O); 6.05 (2d, 2 arom. H); 6.40 (s, 1H, NOH); and 14.15 ppm (s, 1H, OH).

2,4-Dinitrophenylhydrazone Derivative (***9***)—2,4-Dinitrophenylhidrazine (0.25 g), MeOH (5 mL), and H_2SO_4 (0.4 mL) were added to a solution of xanthoxyline (0.1 g) in MeOH (1 mL). The solid formed was recrystallized from chloroform to give **9** (yield, 0.063 g or 33%); mp; 229 °C (lit:[11] 230–231 °C); IR (KBr): 3250, 1600, 1385; and 900 cm^{-1}; ^{1}H-NMR (270 MHz, DMSO-d_6): 2.25 (s, 3H, CH_3); 3.73 (ds, 6H, 2-CH_3O); 7.86 (d, 1 arom. H); 8.40 (2d, 1 arom. H); 8.90 (d, 1 arom. H); 9.70 (s, 1H, NH); 11.00 (s, 1H, OH).

2-Ethyl-3,5-dimethoxyphenyl (***10***)—Xanthoxyline (0.4 g) was dissolved in ethanol (5 mL), and a mixture of amalgamated mossy zinc (1.7 g), water (1.5 mL), and HCl (1.4 g) was added to it. The solution was stirred, refluxed for 40 min, and treated with chloroform. The organic phase was chromatographed on PTLC (hexane: ethyl acetate, 2:1) to give as an oil **10**. (Yield, 0.28 g or 75%); IR (NaCl film): 3400, 3000, 1600, 1370, and 1220 cm^{-1}; ^{1}H-NMR (60 MHz, $CDCl_3$): 1.00 (t, 3H, CH_3); 2.50 (q, 2H, CH_2); 3.60 (ds, 6H, 2-CH_3O); 6.00 (2d, 2 arom. H); and 12.00 ppm (s, 1H, OH).

2-Propoxy-4,6-dimethoxyacetophenone (***11***)—Xanthoxyline (0.2 g), NaOH (0.045 g), MeOH (2 mL), dimethylformamide (DMF, 10 mL),

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and propyl bromide (0.14 g) were refluxed for 1 h and poured into cool water. The crude product was extracted with chloroform and purified by PTLC (hexane:ethyl acetate, 2:1) to give **11** as an oil (yield, 0.1 g or 40%); IR (NaCl film): 3000, 1690, 1600, 1380 cm^{-1}; ^1H-NMR (60 MHz, $CDCl_3$): 1.00 (t, 3H, CH_3); 1.70 (m, 2H, CH_2); 3.70 (s, 3H, CH_3); 3.73 (s, 3H, CH_3O); 3.78 (t, 2H, OCH_2); and 6.00 ppm (2d, 2 arom. H).

2-Acetoxy-4,6-dimethoxyacetophenone ***(12)***—Xanthoxyline (0.1 g) was acetylated in the usual manner with pyridine (1 mL) and acetic anhydrid (0.5 mL). The mixture was refluxed for 5 min and poured into cool water. The resulting precipitate was washed with water to give the pure product **12** (yield, 0.17 g or 70%); mp, 105–107 °C (lit: [12] 106–108 °C); IR (KBr): 3000, 1750, 1660, 1600, and 1380 cm^{-1}; ^1H-NMR (60 MHz, $CDCl_3$): 2.25 (s, 3H, $COCH_3$); 2.50 (s, 3H, $OCOCH_3$); 3.80 (ds, 6H, 2-CH_3O); 6.15 (d, 1 arom. H); and 6.30 ppm (d 1 arom. H).

2-Benzoyloxy-4,6-dimethoxyacetophenone ***(13)***—Xanthoxyline (0.2 g), NaOH (0.045 g), and MeOH (3 mL) were stirred for 15 min, and benzoylchloride (0.2 g) was added. The solution was stirred for another 30 min and poured into 3% HCl (6 mL) containing ~5 g of crushed ice. The product was filtered and purified by PTLC (hexane: ethyl acetate, 2:1) to give **13** (yield, 0.11 g or 38%); mp, 60 °C; IR (KBr): 3000, 1730, 1690, 1400, and 1100 cm^{-1}; ^1H-NMR (60 MHz, $CDCl_3$): 2.40 (s, 3H, CH_3); 3.75 (ds, 6H, 2-CH_3O); 6.30 (2d, 2 arom. H); and 7.50 ppm (m, 5 arom. H).

2-(p-Toluenessulphonyl)-4,6-dimethoxyacetophenone ***(14)***—Xanthoxyline (0.1 g), NaOH (0.03 g), isopropyl alcohol (5 mL), and *p*-toluenessulphonyl chloride (0.12 g) were refluxed for 2 h. The mixture was cooled at room temperature and poured into water. The pure product **14** was extracted with chloroform (yield, 0.08 g or 45%); mp, 150 °C; IR (KBr): 3000, 1680, 1600, and 1355 cm^{-1}; ^1H-NMR (270 MHz, MeOD): 2.25 (s, 3H, CH_3); 2.50 (s, 3H, $COCH_3$); 3.85 (s, 3H, CH_3O); 3.95 (s, 3H, CH_3O); 6.20 (d, 1 arom. H); 6.60 (d, 1 arom. H); 7.50 (2d, 2 arom. H); and 7.80 ppm (2d, 2 arom. H).

2-Benzyloxy-4,6-dimethoxyacetophenone ***(15)***—Xanthoxyline (0.2 g), NaOH (0.045 g), MeOH (2 mL), DMF (10 mL), and benzyl bromide (0.192 g) were refluxed for 1 h. The mixture was cooled at room temperature and poured into water (50 mL). The crude product was extracted with chloroform and purified by PTLC (hexane:ethyl acetate, 2:1) to give **15** (yield, 0.19 g or 64%); mp, 64 °C; IR (KBr): 2990, 1690, 1600, and 1380 cm^{-1}; ^1H-NMR (60 MHz, $CDCl_3$): 2.30 (s, 3H, CH_3); 3.70 (ds, 6H, 2-CH_3O); 4.80 (s, 2H, CH_2); 6.00 (2d, 2 arom. H); and 7.20 ppm (s, 5 arom. H).

2-(4-Methoxybenzyloxy)-4,6-dimethoxyacetophenone ***(16)***—Compound **16** was prepared in manner similar to that described above to yield 0.18 g (56%); mp, 60 °C; IR (KBr): 3000, 1690, 1600, and 1375 cm^{-1}; ^1H-NMR (60 MHz, $CDCl_3$): 2.35 (s, 3H, CH_3); 3.75 (s, 3H, CH_3O); 3.80 (s, 3H, CH_3O); 3.83 (s, 3H, CH_3O); 5.2 (s, 2H, CH_2); 6.00 (d, 1 arom. H); 6.15 (d, 1 arom. H); and 7.35 ppm (s, 4 arom. H).

2-Hydroxy-4,6-dimethoxychalkone ***(17)***—A mixture of xanthoxyline (0.18 g), EtOH (15 mL), NaOH (0.1 g in the minimum of boiled water), and benzaldehyde (0.1 g) was allowed to stand for several hours. The crude product, isolated by acidification of the cooled diluted solution, was recrystallized from hexane to give **17** (yield, 0.135 g or 52%); mp, 84–85 °C; IR (KBr): 3400, 2900, 1625, and 1370 cm^{-1}; ^1H-NMR 60 MHz, $CDCl_3$): 3.98 (s, 3H, CH_3O); 4.00 (s, 3H, CH_3O); 6.20 (d, 1 arom. H); 6.28 (d, 1 arom. H); and 7.80 ppm (m, 5 arom. H); 8.10 (s, 2H, CH=CH).

4',2-dihydroxy-4,6 dimethoxychalkone ***(18)***—Compound **18** was prepared as described above to yield 0.13 g (47%); mp, 193–195 °C (Lit:[13] 194–196 °C); IR (KBr): 3250, 1620, 1600, and 1375 cm^{-1}; ^1H-NMR (200 MHz, DMSO–d_6): 3.80 (s, 3H, CH_3O); 3.90 (s, 3H, CH_3O); 6.13 (2d, 2 arom. H); 6.85–7.60 (m, 4H, arom.); 7.70 (s, 2H, CH=CH); 10.15 (s, 1H, OH); and 13.70 ppm (s, 1H, OH).

Evaluation of Pharmacological Activity Guinea pigs of both sexes (300–500 g) were killed by cervical dislocation and were exaguinated. Preparations of guinea pig ileum (15–20 mm long) were set up in a 5-mL jacketed organ bath containing Tyrode's solution at 37 °C that was bubbled with air under 1 g of load. The Tyrode solution had the following composition (mM): NaCl, (136.8), KCl (2.7), $CaCl_2$ (1.3), $MgCl_2$ (0.5), $NaHCO_3$ (12.0), NaH_2PO_4 (0.14), and glucose (5.5). The isotonic contractions were recorded under 1 g of load with a light lever (sixfold amplification) writing on a kymograph.[14] A stabilization period of at least 60 min was allowed before drug addition, during which the bath solution was renewed every 20 min. All xanthoxyline derivatives were incubated with the preparations for at least 20 min, which in previous experiments proved to be the best contact period. Usually, three to four concentrations of each compound were tested in each preparation. Only one compound was tested in each preparation. After an equilibration period of 40–50 min, sucessive cumulative concentration-response curves were constructed for acetylcholine (0.01–100 μM) at 30–min intervals in the absence or in the presence of increasing concentrations of each compound. The mean of two control contractile responses for acetylcholine was taken to be 100%, and all other responses were calculated as a function of this value. Control experiments for acetylcholine were carried out in parallel in the presence of the vehicle (dimethylsulfoxide or absolute ethanol) used to dilute the test compounds. The final bath concentrations of these solvents did not exceed 0.2% and did not interfere with the tonus of the preparations or with acetylcholine-mediated contraction.

Table 1—Potency (IC_{50}) for Xanthoxyline and Several Derivatives against Acetylcholine–Induced Contraction of the Guinea Pig Ileum in Vitro

Compound	IC_{50}(μM)[a]	Compound	IC_{50}(μM)[a]
1	47 (35–64)[b]	**10**	>500
2	>700	**11**	77 (60–86)
3	>700	**12**	>250
4	407 (223–744)	**13**	>100
5	39 (31–51)	**14**	>300
6	Contractant	**15**	7 (4–12)
7	13 (8–19)	**16**	6 (3–11)
8	>700	**17**	10 (6–18)
9	>700	**18**	8 (5–14)

[a] Each value represents the mean of four to six individual experiments. [b] 95% confidence limits.

The potency of these compounds in atagonizing acetylcholine-mediated contraction was evaluated by estimation of IC_{50} (i.e., the concentrations of compounds required to inhibit the agonist response to 50% relative to control responses). The IC_{50} values were determined for individual experiments, at a minimum of three different concentrations of each compound, with a computer program produced in our laboratory (Armando Dettemer) for least-square regression analysis. Data relative to IC_{50} values are presented as geometric means accompanied by their respective 95% confidence limits ($p < 0.05$).

Results and Discussion

The IC_{50} values for the xanthoxyline derivatives, tested in the guinea pig isolated ileum, against acetylcholine-induced contraction are reported in Table 1. The acetophenone **2** and 4-methoxyacetophenone **3** did not display antispasmodic activity, but dimethoxyacetophenones **4** and especially **5** exhibited potent antispasmodic activity. This fact indicates that two methoxy groups are necessary for this activity. However, **5** was ~10-fold more potent than **4**, indicating that the methoxyl groups in positions 3 and 4 are more effective than those in positions 2 and 4 in producing this activity. It is interesting to note that the substitution pattern of **5** is similar to that found in the aromatic ring of the isoquinolinic moiety of papaverine **7**, where the C=N is compared with the C=O group of **5**.

The transformation of the C=O group into the C=N group of xanthoxyline by synthesis of oxime derivative **8** and the 2,4-dinitrophenylhydrazone derivative **9**, suprisingly, leads to practically inactive compounds. Further studies are needed to determine the reason for this lack of effect considering the similarity of these compounds to papaverine. The importance of the carbonyl group to the xanthoxyline activity is confirmed by the absence of activity of **10**, in which the carbonyl was reduced to CH_2 group. The effects of the substitution of the hydrogen atom of the hydroxyl group in xanthoxyline may be summarized as follows: (i) The introduction of a methyl group gave **6**, which surprisingly displayed marked contractile activity that was not concentration-dependent. In marked contrast, **11**, into which the *n*-propyl group was introduced,

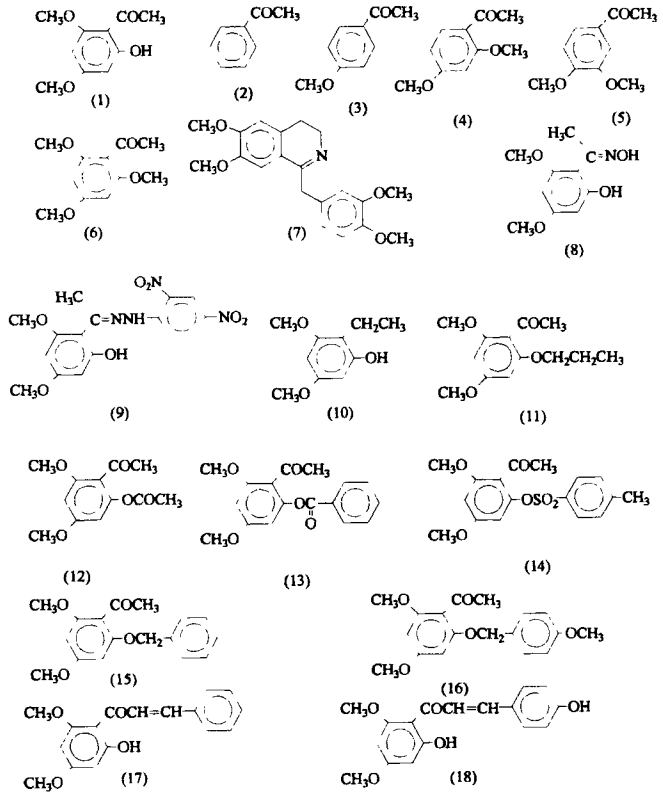

Figure 1—Molecular structures of the studied compounds.

was about two-fold less active than xanthoxyline. (*ii*) The substitution by acetyl and benzoyl groups gave **12** and **13**, respectively, and both exhibited very low antispasmodic activities. A very similar pattern of response was also observed with the tosyl substituent group in **14**. However, the introduction of benzyl and *p*-methoxybenzyl moieties (**15** and **16**, respectively) improved the pharmacological activity; **15** and **16** were about four- to eightfold more potent than xanthoxyline. It should be noted that **13** and **14** have two aromatic groups separated by a polar moiety (−OC=O− and $-OSO_2-$, respectively), whereas **15** and **16** have two aromatic moieties separated by an electronegative atom and an apolar methylene group ($-CH_2-$) similar to papaverine ($-CH_2C=N-$).

Modification of the methyl group bonded to the carbonyl group by condensation with benzaldehyde and *p*-hydroxybenzaldehyde gave **17** and **18**, respectively, which showed higher antispasmodic activity (about fivefold more potent than **1**). These compounds present, like papaverine **7**, two aromatic moieties that are separated by $-C(=O)-C=C-$ instead of $-C(=N)-CH_2$, which results in the two aromatic groups in the same plane.

In summary, our results suggest that both the methoxyl and the carbonyl groups are important factors related to the antispasmodic activity of xanthoxyline and related compounds. In addition, the hydroxyl group enhances, but is not fundamental to the manifestation of the antispasmodic activity. Finally, the introduction of a second aromatic group, like that found in papaverine, explains the great increase in the antispasmodic activity.

These results showed that new active molecules can be designed with active natural products as models. The new molecules should be more specific and active.

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