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Synthesis and Hypolipidaemic Evaluation of a Series of α-Asarone Analogues Related to Clofibrate in Mice

FERNANDO LABARRIOS, LETICIA GARDUÑO*, MARIA DEL ROSARIO VIDAL, RAUL GARCIA, MARIA SALAZAR*, ELIZDATH MARTINEZ*, FRANCISCO DIAZ, GERMAN CHAMORRO AND AND JOAQUIN TAMARIZ·

*Department of Organic Chemistry and *Department of Toxicology, (CQB), National School of Biological Sciences, National Polytechnic Institute, PO Box 105-314, 11591 México, D. F., México*

Abstract

A series of α-asarone analogues related to clofibrate, containing an acetic acid group at C-2 of the aromatic ring, has been prepared as the acids or as the ethyl and methyl esters. The corresponding alcohols were also synthesized by reduction of the ethyl esters. The compounds were examined in hyperlipidaemic male mice to evaluate their ability to modify serum lipoprotein cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides after oral administration of 40 and 80 mg kg^{-1} for 6 days.

Except for methyl 2-methoxy-5-nitro-4-(2-propenyl)phenoxyacetate at either dose, these clofibrate-related phenoxyacetic acid derivatives were found to have significant hypocholesterolaemic activity. Levels of low-density lipoprotein cholesterol and triglycerides were significantly reduced and those of high-density lipoprotein cholesterol were elevated. 2-Methoxy-5-nitro-4-(2-propenyl)phenoxyacetic acid was active at both doses in all the tests. Clofibrate (150 mg kg^{-1}) was more potent at reducing low-density lipoprotein cholesterol. No activity was detected for the alcohol derivatives.

These preliminary results suggest that this class of compound might have more promise as potential hypolipidaemic agents than other α-asarone derivatives. Further investigation and characterization should be performed to determine the mode of action of these agents on lipid metabolism.

α-Asarone (Figure 1), the active principle of the native Mexican plant *Guatteria gaumeri* (annonaceae), has previously been shown to be a potent hypolipidaemic agent in rodents after oral administration (Gómez et al 1987; Garduño et al 1997). Moreover, exposure of 3T3-hepatocyte cultures to micromolar concentrations of α-asarone significantly inhibited lipid secretion and probably lipid synthesis, suggesting that at least part of the hypolipidaemic effect could be a result of reduction in the secretion of lipids by the hepatocytes (Hernández et al 1993). A toxicity study on long-term cultures of adult rat hepatocytes revealed morphological and structural alterations, triacylglycerol accumulation and inhibition of protein synthesis and secretion (López et al 1993). The secretion of proteins was the most sensitive indication of toxicity. Mutagenic (Morales-Ramírez et al 1992) and teratogenic (Salazar et al 1992) effects have been also demonstrated in mice. These results prompted us to undertake the synthesis and evaluation of several non-conjugated dimethoxypropenyl derivatives of α-asarone (Muñoz et al 1993; Chamorro et al 1998). Results of experiments with mice revealed hypolipidaemic reduction of lipid levels in the serum, suggesting that the lack of conjugation of the propenyl chain with the aromatic ring does not significantly affect the pharmacological profile of these α-asarone analogues.

With the aim of establishing a structure–activity relationship for this class of promising hypolipidaemic agent, in this paper we address the unexplored role played by the C-2 methoxy group in the activity of these compounds. We now present the synthesis of clofibrate (**1**)-related phenoxyacetic derivatives **2a** and **2b**, and their corresponding methyl and ethyl esters **2c**–**2f**. The biological evaluation of these compounds and the alcohol derivatives **3a**–**3c** is also reported.

Correspondence: J. Tamariz or G. Chamorro, National School of Biological Sciences, PO Box 105-314, 11591 México, D. F., Mexico.

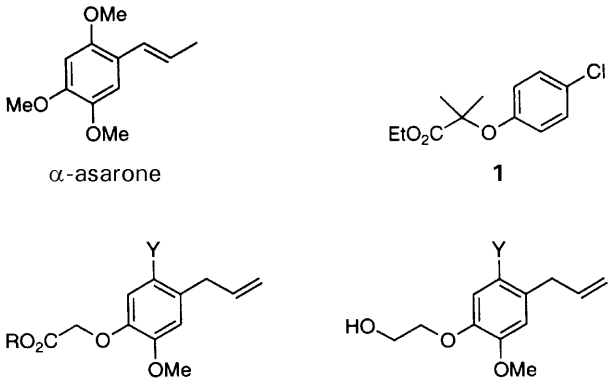

Figure 1. Chemical structures of α-asarone, clofibrate (**1**), clofibrate-related phenoxyacetic derivatives (**2a** and **2b**), the corresponding methyl and ethyl esters (**2c**–**2f**), and the alcohol derivatives (**3a**–**3c**).

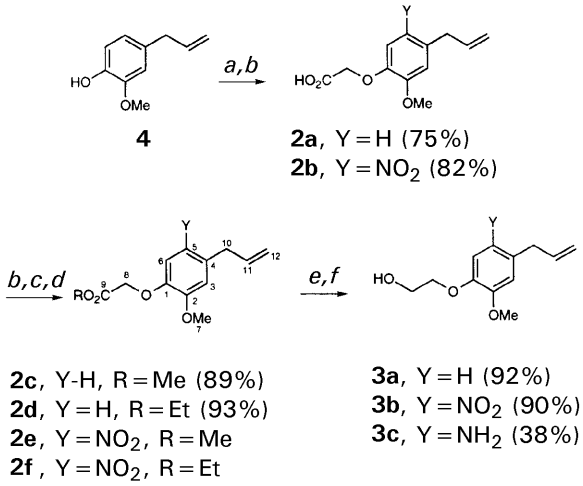

Figure 2. Synthesis of compounds **2a**–**2f** and **3a**–**3c**. (a) aqueous NaOH, **5**, 50°C, 7 h; (b) HNO_3, acetic acid, 5°C, 1 h; (c) methanol, HCl, reflux, 4 h; (d) ethanol, HCl, reflux, 4 h; (e) $LiAlH_4$, THF, reflux, 4 h; (f) $Na_2S_2O_4$, methanol.

Materials and Methods

Synthesis

Starting materials and reagents were purchased from Aldrich and were used without further purification. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} plates (Merck); short- and long-wavelength ultraviolet light were used to visualize the spots.

Melting points were determined with a Thomas-Hoover melting-point apparatus and are uncorrected. Infrared (IR) spectra were obtained with a Perkin-Elmer 599B spectrophotometer. ^1H and ^{13}C Nuclear magnetic resonance (NMR) spectra were recorded with a Varian Gemini (300 MHz) spectrometer; TMS was used as internal standard. Mass spectra were acquired with a Hewlett-Packard 5971A spectrometer. Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ).

The preparation of compounds **2** was performed as depicted in Figure 2. Basic treatment of **4** in the presence of chloroacetic acid (**5**) furnished 2-methoxy-4-(2-propenyl)phenoxyacetic acid (**2a**) in 75% yield. Nitration of **2a** under mild conditions gave a satisfactory yield of **2b**. The methyl and ethyl esters **2c** and **2d** were prepared in high yields by acidic treatment of **2a** with methanol and ethanol, respectively. When these esters were subjected to nitration under similar conditions, the nitro compounds **2e** (53%) and **2f** (40%) were obtained in moderate yields.

To improve the preparation of compounds **2e** and **2f**, an alternative approach was followed. By esterification of nitro compound **2b**, under acidic conditions (gaseous HCl, under reflux) the corresponding alcohol derivatives, **2e** and **2f** were isolated as pale yellow crystals in better yields (83 and 68%, respectively).

The reduction of esters **2d** and **2f** by treatment with lithium aluminium hydride furnished alcohols **3a** and **3b**, respectively, in good yields (Figure 2). The amino compound **3c** was obtained by reduction of the nitro alcohol **3b** with sodium hydrosulphite (Díaz et al 1991, 1993).

Synthesis of 2-methoxy-4-(2-propenyl)phenoxyacetic acid, **2a**

An aqueous solution of NaOH (4·49 g, 0·112 mol) and chloroacetic acid (**5**) (7·01 g, 0·074 mol) in water were successively added dropwise to compound **4** (10·65 g, 0·065 mol) at room temperature, keeping the temperature of the reaction mixture below 60°C. The mixture was maintained without stirring for 1 h at room temperature and then stirred at 50°C for 7 h. A concentrated aqueous solution of HCl (5 mL) was added and the mixture was filtered. Hexane was then slowly added to this solution until crystallization. The crystals were removed by filtration and recrystallized from hexane, giving 10·81 g (75%) **2a** as colourless needles: R_F 0·84 ($CHCl_3$-C_2H_5OH, 1 : 1); mp 98–99°C (Abraham et

al 1984, 95–97°C); IR (KBr) 3520, 3400, 3200–2400, 1750, 1520, 1255, 1230, 1160, 1030, 910, 810 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6·83 (dm, J = 8·0 Hz, 1H, H-6), 6·75 (d, J = 1·9 Hz, 1H, H-3), 6·72 (dd, J = 8·0, 1·9 Hz, 1H, H-5), 6·40 (br, 1H, CO_2H), 5·94 (m, 1H, $ArCH_2CH=$), 5·08 (dm, J = 16·0 Hz, 1H, $CH_2=$), 5·07 (dm, J = 11·0 Hz, 1H, $CH_2=$), 4·68 (s, 2H, CH_2O), 3·87 (s, 3H, CH_3O), 3·33 (dm, J = 6·7 Hz, 2H, $ArCH_2CH=$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 40·0 (C-10), 56·0 (C-7), 67·6 (C-8), 112·8 (C-3), 116·1 (C-12), 116·3 (C-6), 121·0 (C-5), 135·8 (C-4), 137·2 (C-11), 145·6 (C-1), 149·6 (C-2), 172·6 (C-9); MS (70 eV) m/z 222 (M^+, 100), 207 (1), 163 (40), 147 (7), 131 (11), 115 (13), 107 (17), 103 (28), 91 (22), 77 (12). Analysis: calc. for $C_{12}H_{14}O_4$: C, 64·86; H, 6·30; found: C, 64·67; H, 6·21.

Synthesis of 2-methoxy-5-nitro-4-(2-propenyl)phenoxyacetic acid, **2b**

Conc. HNO_3 (3·5 mL, 0·082 mol) was added dropwise to a solution of **2a** (6·98 g, 0·0314 mol) in glacial acetic acid (25 mL, 0·437 mol) at 5°C, keeping the temperature constant. The mixture was stirred for 1 h and poured on to ice (100 g). The precipitate was removed by filtration, washed with water (3 × 20 mL), and recrystallized from hexane-ethyl acetate, giving 6·88 g (82%) **2b** as a pale brown powder: R_F 0·79 (hexane-ethyl acetate, 7 : 3); mp 105–106°C; IR (KBr) 3500–2400, 1740, 1590, 1520, 1335, 1280, 1205, 1086, 1050, 930, 860, 795 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7·62 (s, 1H, H-6), 6·95 (br, 1H, CO_2H), 6·80 (s, 1H, H-3), 5·98 (ddt, J = 17·0, 10·2, 6·4 Hz, 1H, $ArCH_2CH=$), 5·13 (ddt, J = 10·2, 1·5, 1·4 Hz, 1H, $CH_2=$), 5·10 (ddt, J = 17·0, 1·6, 1·5 Hz, 1H, $CH_2=$), 4·76 (s, 2H, OCH_2), 3·98 (s, 3H, OCH_3), 3·74 (dm, J = 6·4 Hz, 2H, $ArCH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 37·5 (C-10), 56·4 (C-7), 66·1 (C-8), 111·5 (C-6), 113·9 (C-3), 117·1 (C-12), 132·2 (C-4), 135·1 (C-11), 141·0 (C-5), 145·0 (C-1), 153·5 (C-2), 172·2 (C-9). Analysis: calc. for $C_{12}H_{13}NO_6$: C, 53·93; H, 4·90; N, 5·24; found: C, 53·73; H, 5·03; N, 5·29.

Synthesis of methyl 2-methoxy-4-(2-propenyl)phenoxyacetate, **2c**

Compound **2a** (2·60 g, 0·0117 mol) was mixed with a saturated methanolic solution of gaseous HCl (5 mL) at room temperature. The mixture was heated to reflux for 4 h. The solvent was removed in-vacuo and the residue was crystallized in hot hexane. After recrystallization (hexane), 2·46 g (89%) of **2c** were obtained as colourless crystals: R_F 0·41 (hexane-ethyl acetate, 7 : 3); mp 42·5–44°C; IR (CH_2Cl_2) 1760, 1637, 1593, 1512, 1425, 1281, 1263, 1246, 1207, 1146, 1134, 918, 784, 680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6·76 (d, J = 8·1 Hz, 1H, H-6), 6·73 (d, J = 1·9 Hz, 1H, H-3), 6·68 (dd, J = 8·1, 1·9 Hz, 1H, H-5), 5·94 (ddt, J = 16·8, 10·2, 6·7 Hz, 1H, $ArCH_2CH=$), 5·09 (dm, J = 16·8 Hz, 1H, *Z*-$CH_2=$), 5·07 (dm, J = 10·2 Hz, 1H, *E*-$CH_2=$), 4·67 (s, 2H, OCH_2), 3·86 (s, 3H, OCH_3), 3·78 (s, 3H, CO_2CH_3), 3·33 (da, J = 6·7 Hz, 2H, $ArCH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 40·0 (C-11), 52·2 (C-10), 55·9 (C-7), 67·1 (C-8), 113·0 (C-3), 115·0 (C-6), 116·0 (C-13), 121·8 (C-5), 134·9 (C-4), 137·5 (C-12), 145·8 (C-1), 149·8 (C-2), 170·0 (C-9); MS (70 eV) m/z 236 (M^+, 100), 221 (1), 209 (2), 177 (12), 163 (56), 131 (22), 115 (26), 103 (42), 91 (37), 77 (20). Analysis: calc. for $C_{13}H_{16}O_4$: C, 66·09; H, 6·82; found: C, 66·16; H, 6·71.

Synthesis of ethyl 2-methoxy-4-(2-propenyl)phenoxyacetate, **2d**

The same procedure as for **2c** was used, but with a saturated ethanolic solution of gaseous HCl, to give 2·74 g (93%) **2d** as colourless needles: R_F 0·5 (hexane-ethyl acetate, 7 : 3); mp 34–35°C; IR (CH_2Cl_2) 1759, 1639, 1592, 1512, 1464, 1434, 1295, 1279, 1246, 1200, 1146, 1073, 1034, 918, 784 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6·77 (d, J = 8·2 Hz, 1H, H-6), 6·74 (d, J = 1·8 Hz, 1H, H-3), 6·69 (d, J = 8·2, 1·8 Hz, 1H, H-5), 5·95 (ddt, J = 16·7, 10·2, 6·6 Hz, 1H, $ArCH_2CH=$), 5·09 (ddt, J = 16·7, 3·4, 1·4 Hz, 1H, *Z*-$CH_2=$), 5·07 (ddt, J = 10·2, 3·3, 1·4 Hz, 1H, *E*-$CH_2=$), 4·67 (s, 2H, OCH_2), 4·26 (q, J = 7·1 Hz, 2H, CO_2CH_2), 3·86 (s, 3H, OCH_3), 3·34 (br d, J = 6·6 Hz, 2H, $ArCH_2$), 1·28 (t, J = 7·1 Hz, 3H, $COCH_2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14·2 (C-11), 39·8 (C-12), 55·9 (C-7), 61·2 (C-10), 67·0 (C-8), 112·8 (C-3), 114·9 (C-6), 115·8 (C-14), 120·5 (C-5), 134·6 (C-4), 137·5 (C-13), 145·8 (C-1), 149·7 (C-2), 169·2 (C-9); MS (70 eV) m/z 250 (M^+, 100), 235 (1), 223 (2), 178 (19), 163 (85), 147 (8), 131 (30), 115 (29), 103 (46), 91 (36). Analysis: calc. for $C_{14}H_{18}O_4$: C, 67·18; H, 7·25; found: C, 67·30; H, 7·27.

Synthesis of methyl 2-methoxy-5-nitro-4-(2-propenyl)phenoxyacetate, **2e**

Method A. Compound **2b** (4 g, 0·015 mol) was mixed with a saturated methanolic solution of gaseous HCl (20 mL) at room temperature and the mixture was heated to reflux for 4 h. The solvent was removed in-vacuo and the residue was crystallized by dissolving it in the minimum amount of

ethyl acetate, cooling to 0°C and adding hexane dropwise. Recrystallization was performed similarly, giving 3·5 g (83%) **2e** as pale yellow crystals.

Method B. Conc. HNO_3 (2·64 g, 0·042 mol) was added dropwise to a solution of **2c** (5·0 g, 0·0212 mol) in glacial acetic acid (25 mL, 0·437 mol) at 5°C (prepared by adding acetic acid dropwise to **2c** at 5°C and stirring the mixture for 15 min at the same temperature), keeping the temperature constant. The mixture was stirred for 1 h and poured on to ice (100 g). The precipitate was filtered, washed with water (3 × 20 mL) and recrystallized from ethyl acetate-hexane, giving 3·15 g (53%) **2e** as pale yellow crystals: R_F 0·32 (hexane-ethyl acetate, 7 : 3); mp 107–109°C; IR (CH_2Cl_2) 3060, 1761, 1637, 1583, 1519, 1441, 1334, 1261, 1211, 1180, 1081, 1051, 998, 923, 868, 793 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7·57 (s, 1H, H-6), 6·78 (s, 1H, H-3), 5·98 (ddt, J = 16·9, 10·2, 6·5 Hz, 1H, $ArCH_2CH=$), 5·12 (ddt, J = 10·2, 1·5, 1·4 Hz, 1H, *E*-$CH_2=$), 5·09 (ddt, J = 16·9, 1·6, 1·5 Hz, 1H, *Z*-$CH_2=$), 4·74 (s, 2H, OCH_2), 3·96 (s, 3H, OCH_3), 3·82 (s, 3H, CO_2CH_3), 3·73 (dm, J = 6·5 Hz, 2H, $ArCH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 37·8 (C-11), 52·6 (C-10), 56·4 (C-7), 66·1 (C-8), 111·0 (C-6), 114·0 (C-3), 117·1 (C-13), 130·9 (C-4), 135·2 (C-12), 141·0 (C-5), 145·4 (C-1), 153·6 (C-2), 168·4 (C-9); MS (70 eV) m/z 281 (M^+, 17), 264 (100), 251 (22), 222 (15), 204 (20), 190 (18), 174 (25), 162 (34), 146 (15), 133 (21), 115 (29), 91 (21), 77 (21), 55 (25). Analysis: calc. for $C_{13}H_{15}NO_6$: C, 55·51; H, 5·37; found: C, 55·58; H, 5·20.

NMR and IR spectroscopy and melting-point determination showed that the products obtained by methods A and B were identical.

Synthesis of ethyl 2-methoxy-5-nitro-4-(2-propenyl)phenoxyacetate, **2f**

Method A. The procedure followed was the same as for **2e** (method A) but **2b** was used. A saturated ethanolic solution of gaseous HCl gave 3·0 g (68%) **2f** as pale yellow crystals.

Method B. The same procedure as for **2e** (method B) was used, but with **2d** (5·0 g, 0·02 mol) and 2·52 g (0·04 mol) conc. HNO_3, yielding 2·36 g (40%) **2f** as pale yellow crystals: R_F 0·40 (hexane-ethyl acetate, 7 : 3); mp 54–55°C; IR (CH_2Cl_2) 3060, 1752, 1637, 1612, 1583, 1524, 1463, 1437, 1332, 1294, 1203, 1050, 1021, 996, 878 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7·57 (s, 1H, H-6), 6·78 (s, 1H, H-3), 5·98 (ddt, J = 16·9, 10·2, 6·5 Hz, 1H, $ArCH_2CH=$), 5·13 (ddt, J = 10·2, 1·6, 1·4 Hz, 1H, *E*-$CH_2=$), 5·09 (ddt, J = 16·9, 1·65, 1·60 Hz, 1H, *Z*-$CH_2=$), 4·72 (s, 2H, OCH_2), 4·28 (q, J = 7·1 Hz, 2H, $CO_2CH_2CH_3$), 3·96 (s, 3H, OCH_3), 3·72 (dm, J = 6·5 Hz, 2H, $ArCH_2$), 1·31 (t, J = 7·1 Hz, 3H, $CO_2CH_2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14·2 (C-11), 37·6 (C-12), 56·4 (C-7), 61·6 (C-10), 66·3 (C-8), 110·8 (C-6), 113·8 (C-3), 117·0 (C-14), 131·6 (C-4), 135·2 (C-13), 140·9 (C-5), 145·4 (C-1), 153·6 (C-2), 168·0 (C-9); MS (70 eV) m/z 295 (M^+, 20), 278 (100), 265 (22), 250 (21), 222 (40), 208 (24), 204 (22), 190 (23), 174 (23), 162 (36), 115 (32), 91 (25), 77 (27), 55 (23). Analysis: calc. for $C_{14}H_{17}NO_6$: C, 56·95; H, 5·80; found: C, 57·12; H, 5·61.

Synthesis of 1-(2-hydroxyethyl)-2-methoxy-4-(2-propenyl)benzene, **3a**

2d (1·0 g, 4·0 mmol) was dissolved in dry THF (50 mL) in a two-necked round-bottom flask. The solution was stirred and heated to reflux and $LiAlH_4$ (0·76 g, 20 mmol) was slowly added (2 h). The mixture was heated to reflux for a further 2 h, then filtered, and the residue was washed with aqueous NaOH (10%, 10 mL) and the aqueous filtrate extracted with CH_2Cl_2 (3 × 10 mL). The original filtrate was concentrated in-vacuo, and aqueous NaOH (10%, 10 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 10 mL). The organic phases were mixed, dried (Na_2SO_4) and evaporated in-vacuo, giving a white solid residue which was recrystallized (hexane) to give 0·76 g (92%) **3a** as colourless crystals. R_F 0·57 (hexane-acetone, 6 : 4); mp 38–40°C; IR (KBr) 3390, 1508, 1259, 1131, 1075, 1027 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6·86 (d, J = 8·2 Hz, 1H, H-6), 6·78 (d, J = 1·7 Hz, 1H, H-3), 6·67 (dd, J = 8·2, 1·7 Hz, 1H, H-5), 5·94 (ddt, J = 16·8, 10·0, 6·8 Hz, 1H, $ArCH_2CH=$), 5·14–5·00 (m, 2H, $CH_2=$), 4·59 (br s, 1H, OH), 3·92 (t, J = 4·9 Hz, 2H, CH_2OAr), 3·74 (s, 3H, CH_3O), 3·74–3·69 (m, 2H, CH_2OH), 3·29 (br d, J = 6·6 Hz, 2H, CH_2Ar); ^{13}C NMR (75 MHz, DMSO-d_6) δ 39·1 (C-10), 55·4 (C-7), 59·6 (C-9), 70·3 (C-8), 112·5 (C-3), 113·4 (C-6), 115·5 (C-12), 120·2 (C-5), 132·4 (C-4), 138·0 (C-11), 146·5 (C-1), 148·9 (C-2). MS (70 eV) m/z 208 (M^+, 42), 164 (68), 149 (65), 131 (57), 115 (30), 103 (71), 78 (87), 77 (100), 65 (52), 55 (77). Analysis: calc. for $C_{12}H_{16}O_3$: C, 69·21; H, 7·74; found: C, 69·38; H, 7·51.

Synthesis of 1-(2-hydroxyethyl)-2-methoxy-5-nitro-4-(2-propenyl)benzene, **3b**

The same procedure as for **3a** was used, but with **2f** (1·0 g, 3·4 mmol) and 0·64 g (17 mmol) $LiAlH_4$ to give 0·77 g (90%) **3b** as pale yellow crystals: R_F

0·52 (hexane-ethyl acetate, 8 : 2); mp 90–91°C; IR (KBr) 3350, 1512, 1328, 1270, 1230, 1073 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7·64 (s, 1H, H-6), 6·75 (s, 1H, H-3), 5·98 (ddt, J = 16·9, 10·1, 6·7 Hz, 1H, ArCHC*H*=), 5·15–5·00 (m, 2H, CH_2=), 4·17 (t, J = 4·4 Hz, 2H, CH_2OAr), 4·00 (t, J = 4·4 Hz, 2H, CH_2OH), 3·94 (s, 3H, CH_3O), 3·72 (br d, J = 6·4 Hz, 2H, $ArCH_2$), 2·90-2·65 (br, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 37·5 (C-10), 56·2 (C-7), 60·9 (C-9), 71·0 (C-8), 110·3 (C-6), 113·3 (C-3), 116·8 (C-12), 130·7 (C-4), 135·3 (C-11), 141·0 (C-5), 146·3 (C-1), 153·4 (C-2); Analysis: calc. for $C_{12}H_{15}NO_5$: C, 56·91; H, 5·97; N, 5·53; found: C, 56·77; H, 5·76; N, 5·45.

Synthesis of 5-amino-1-(2-hydroxyethyl)-2-methoxy-4-(2-propenyl)benzene, **3c**

3b (2·0 g, 7·9 mmol) was dissolved in methanol (50 mL) by heating to reflux in a two-necked round-bottom flask. A solution of $Na_2S_2O_4$ (5·22 g, 30 mmol) in H_2O (30 mL) was added dropwise, and the mixture was heated for 2 h. The solution was filtered and the residue was washed with CH_2Cl_2 (2 × 10 mL). The filtrate was separated into two phases and the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The organic phase was dried (Na_2SO_4) and evaporated in-vacuo, giving dark brown crystals, which were recrystallized (hexane) to give 0·67 g (38%) **3c**, as pale brown crystals. R_F 0·54 (hexane-acetone, 1 : 1); mp 84–85°C; IR (KBr) 3351, 1508, 1259, 1203, 1123, 1083, 1000 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6·62 (s, 1H, H-3), 6·12 (s, 1H, H-6), 5·92 (ddt, J = 16·6, 10·2, 6·1 Hz, 1H, ArCHC*H*=), 5·11 (ddt, J = 10·2, 1·7, 1·5 Hz, 1H, *E*-CH_2=), 5·07 (ddt, J = 17·0, 1·7, 1·6 Hz, 1H, *Z*-CH_2=), 4·06–4·03 (m, 2H, CH_2O), 3·89–3·86 (m, 2H, C*H*$_2$COOH), 3·79 (s, 3H, OCH_3), 3·25 (ddd, J = 6·1, 1·6, 1·5 Hz, 2H, $ArCH_2$), 3·20–3·10 (br, 3H, NH_2, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 36·0 (C-10), 56·9 (C-7), 61·3 (C-9), 71·9 (C-8), 105·1 (C-6), 115·4 (C-3), 115·9 (C-12), 117·4 (C-4), 135·9 (C-11), 138·7 (C-5), 143·1 (C-2), 147·5 (C-1); MS (70 eV) m/z 223 (M^+, 88), 208 (14), 164 (100), 150 (15), 136 (61), 118 (18), 91 (13), 77 (8). Analysis: calc. for $C_{12}H_{17}NO_3$: C, 64·55; H, 7·67; N, 6·27; found: C, 64,71; H, 7·62; N, 6·33.

Biological screening

Experiments were performed on CF1 male mice, 25–28 g, from the Instituto Nacional de Virología, Secretaría de Salud, Mexico City. The animals were housed in hanging metal cages (two in each) and maintained at a temperature of 24 ± 2°C, 45% relative humidity and 12-h periods of light and darkness. A high-cholesterol diet (cholesterol 1·0%, sodium cholate 0·5%, butter 5%, sucrose 30·0%, casein 10%, laboratory chow 53·5%, prepared from a powdered basal diet; Lab Rodent Diet 5001) was made freely available for 6 days (Matsuda et al 1986). Mice fed with laboratory chow for the same duration as above were used as non-cholesterol control groups. Compounds, dissolved in 1 : 9 Tween 80-water, were administered orally by an intubation needle at doses of 40 and 80 mg kg^{-1} once a day for the duration of the experiment. Concentrations were adjusted so that a 30-g mouse would receive 0·15 mL. Mice in the control group received a similar volume of the vehicle. At the end of 6 days, each animal was fasted for 12–15 h. Blood samples were collected by means of an orbital bleeding technique and centrifuged at 3000 *g* for 10 min. After blood collection the animals were killed and inspected for gross abnormalities. Total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were determined by means of an Abbott VP Bichromatic Analyzer.

All data are expressed as means ± s.d. (standard deviation) and were analysed statistically by Student's *t*-test (Tallarida & Murray 1987). *P* values less than 0·05 were considered as indicative of significance.

Results and Discussion

Owing to interest in the synthesis of new hypolipidaemic agents and as a continuation of our research (Díaz et al 1993; Muñoz et al 1993) we designed and synthesized a new series of α-asarone analogues and evaluated their pharmacological activity. Table 1 list details of data on the lipid-reducing activity of these compounds.

All animals appeared healthy throughout the dosing period, maintaining normal food intake. No gross abnormalities were observed in any treated mice. Treatment of mice with the hyperlipidaemic diet for 6 days resulted in a significant elevation in total serum cholesterol (from 3·4 ± 0·2 to 8·6 ± 0·4 mM) and LDL cholesterol (from 0·4 ± 0·1 to 7·5 ± 0·3 mM), reduction of HDL cholesterol (from 2·8 ± 0·3 to 1·4 ± 0·1 mM) and total triglycerides (from 1·4 ± 0·8 to 1·4 ± 0·4 mM). Clofibrate (**1**), the reference antihyperlipidaemic drug, at a dose of 150 mg kg^{-1} day^{-1}, reduced cholesterol by 39·5%, LDL cholesterol by 49·9%, triglycerides by 20·1%, and HDL by 14·6%.

The reduced cholesterol levels were accompanied by a concomitant reduction in LDL cholesterol and triglyceride levels. Triglyceride levels were also reduced by both doses of the alcohol derivative **3b**.

Table 1. Hypolipidaemic effect of derivatives of α-asarone

Compound	Dose (mg mg − 1)	Total cholesterol	Low-density lipoprotein cholesterol	High-density lipoprotein cholesterol	Triglycerides
Normal diet	–	−60·5 ± 0·2†	−7·6 ± 0·08	+ 103·6 ± 0·28†	−94·9 ± 0·11†
Cholesterol diet	–	100 ± 0·4‡	100 ± 0·04§	100 ± 0·11¶	−100 ± 0·30**
2a + cholesterol diet	40	−24·4 ± 0·3†	−18·7 ± 0·06†	+ 41·6 ± 0·06†	−48·1 ± 0·25†
	80	−30·2 ± 0·2†	−37·5 ± 0·06†	+ 33·6 ± 0·23	−54·9 ± 0·48†
2b + cholesterol diet	40	−17·4 ± 0·2†	−25·0 ± 0·04†	+ 46·7 ± 0·11†	−43·3 ± 0·39†
	80	−41·9 ± 0·3†	−13·9 ± 0·03†	+ 91·2 ± 0·27†	−65·9 ± 0·46†
2c + cholesterol diet	40	−23·2 ± 0·5*	−17·4 ± 0·08*	+ 45·2 ± 0·06†	−52·7 ± 0·82†
	80	−53·5 ± 0·2†	−25·7 ± 0·02†	+ 12·4 ± 0·25	−51·6 ± 0·61†
2d + cholesterol diet	40	−41·9 ± 0·3†	−21·5 ± 0·02†	−48·0 ± 0·13†	−11.1 ± 1·10
	80	−26·7 ± 0·2†	−15·3 ± 0·04†	−25.5 ± 0·09*	−45·7 ± 0·39*
2e + cholesterol diet	40	−12·8 ± 0·4	−19·4 ± 0·03†	+ 89·0 ± 0·38†	−33·1 ± 0·44†
	80	−17·4 ± 0·6	−26·4 ± 0·00†	+ 98·5 ± 0·71*	−35·2 ± 1·31*
2f + cholesterol diet	40	−41·9 ± 0·3†	−11·1 ± 0·04*	+ 28·5 ± 0·09*	−53·9 ± 0·56†
	80	−48·8 ± 0·4†	−28·5 ± 0·02†	−18·9 ± 0·16	−45·4 ± 0·91†
3a + cholesterol diet	40	−15·1 ± 0·81	−23·1 ± 0·04†	−15·3 ± 0·09	−25·1 ± 0·84
	80	+ 15·1 ± 0·60	−30·5 ± 0·02†	−45·9 ± 0·08†	+ 2·27 ± 0·59
3b + cholesterol diet	40	−4·6 ± 0·6	−6·2 ± 0·03	−0·00 ± 0·06	−28·7 ± 0·68*
	80	+ 3·9 ± 0·6	−9·7 ± 0·08	+ 30·6 ± 0·27	−42·5 ± 0·87*
3c + cholesterol diet	40	−4·6 ± 0·9	+ 4·2 ± 0·05	+ 27·0 ± 0·23	−12·2 ± 1·32
	80	+ 40·7 ± 0·4†	+ 6·25 ± 0·06	+ 8·32 ± 0·12	+ 14·30 ± 0·80
Clofibrate (**1**)	150	−39·5 ± 0·06†	−49·9 ± 0·53†	−14·6 ± 0·90†	−20·1 ± 0·06†

Data (mean ± standard error; n = 6) are expressed as percentages of the result for the cholesterol diet group. * $P < 0.05$, † $P < 0.01$ significantly different from result for cholesterol diet group. ‡ 8·6 mM, § 7·5 mM; ¶ 1·4 mM; ** 1·4 mM.

A statistically significant increase in HDL cholesterol was discerned after dosing with compounds **2a**–**2c**, **2e** and **2f**. Analogue **2b** was active at 40 and 80 mg kg^{-1} day^{-1} in all tests.

Clofibrate-related phenoxyacetic derivatives and their corresponding methyl and ethyl esters, **2a**–**2f**, administered orally at doses of 40 and 80 mg kg^{-1} day^{-1}, proved to be effective hypolipidaemic agents in mice. In hyperlipidaemic induced mice the drugs reduced the elevated levels of serum cholesterol, LDL cholesterol and triglycerides and increased the HDL cholesterol. At 80 mg kg^{-1} day^{-1} the methyl ester **2c** was more active than ethyl ester **2f** or the acid **2b**. The clofibrate-related phenoxyacetic derivative **2a** and its ethyl ester **2d** also had significant hypocholesterolaemic activity. Exceptions were compound **2e**, which reduced total cholesterol, but not significantly, and compound **2d**, which also reduced HDL cholesterol. However, these compounds were active in the other tests.

The hypocholesterolaemic effect of compounds **2b**, **2c** and **2f** was comparable with or greater than that of clofibrate (**1**) at its therapeutic effective dose of 150 mg kg^{-1} day^{-1}. This difference was more important for triglycerides, which all compounds except **2d** reduced by a larger amount after a 40 mg kg^{-1} day^{-1} dose. Compound **1** was more effective at reducing LDL cholesterol than our synthesized compounds.

The alcohol derivatives **3a**–**3c** did not have consistent hypolipidaemic activity, suggesting that the carboxylic group in compounds **2** is involved in their activity. In addition, and in agreement with previous results (Chamorro et al 1998), the hypolipidaemic activity of compounds **2a**–**2f**, which have the unconjugated propenyl group, also suggests that conjugation of the double bond of the side-chain with the aromatic ring is not an important factor in determining the high hypolipidaemic activity shown by α-asarone and some of its 4-substituted derivatives (Díaz et al 1993).

Various genetic and environmental factors contribute to the development and progression of atherosclerotic disease (Dart et al 1997). Elevated blood cholesterol has been conclusively established in epidemiological studies as a major independent risk factor for coronary heart disease and other vascular diseases (Byington et al 1995); in hyperlipidaemic patients who suffer from myocardial infarction or atherosclerosis, or both, LDL cholesterol is high and HDL cholesterol low (Hall et al 1987; Chapman et al 1989). This relationship favours the movement of cholesterol to peripheral tissue, including atherogenic plaques, where cholesterol ester accumulates (Hall et al 1987).

The current results also showed that repeated administration of these α-asarone analogues, containing an acetic acid group on C-2 of the aromatic ring, to hyperlipidaemic mice reduced triglyceride levels. Although the impact of triglyceridaemia on cardiovascular disease is controversial, recent studies have suggested that it might contribute to cardiovascular disease by increasing the risk in

patients who already have an adverse LDL/HDL ratio (Hughes et al 1995).

The compounds synthesized are members of a unique structural series which seem to have hypolipidaemic activity. The role of different substituents in eliciting the observed activity might not be rationalized until the mechanism of action is known, a determination which was beyond the scope of this investigation. However, on the basis of a previous study of α-asarone which suggested that at least part of its hypolipidaemic effect could be because of a reduction in the secretion of lipids by hepatocytes (Hernández et al 1993), a similar mechanism of action might be expected of these derivatives.

Thus, this class of potential hypolipidaemic agents warrants further investigation and characterization with regard to the mode of action of the compounds on lipid metabolism, because the results of this preliminary study imply that they might have more promise as a chemically useful agent than other α-asarone derivatives

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