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Novel stereoselective synthesis and chromatographic evaluation of *E*-guggulsterone

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ABSTRACT

A new stereoselective synthesis of *E*-guggulsterone is described starting from androsten-3,17-dione. Protection of the ring A enonic system, followed by regioselective Wittig reaction and C-16 oxidation, affords *E*-guggulsterone in good yields and high stereoselectivity, making this approach easily accessible and scalable. Moreover, an original normal-phase HPLC method enabling the fast quantitation of the guggulsterone isomeric purity, combined with the suitability for sampling procedures, is detailed. The relying upon the cellulose-based Chiralpak IB column and the chloroform as the "non-standard" component of the eluent mixture, allows to get profitably high chromatographic performances.

1. Introduction

The herbal extract guggulipid from the tree *Commiphora mukul* has been used in Indian Ayurvedic medicine for more than 2000 years to treat a variety of ailments including diabetes, atherosclerosis, as well as osteoarthritis and inflammation [1,2]. The gum resin, which is available on the market since 1988 as a potent hypolipidaemic agent, is a complex mixture of diverse classes of compounds, such as lignans, lipids, diterpenoids and steroids [3]. Among these, two steroidal isomers known as *E*- and *Z*-guggulsterone (*E*- and *Z*-4,17(20)-pregnadiene-3,16-dione, **1** and **2**) (Fig. 1) are considered as the key active ingredients responsible for guggul's therapeutic effects. Guggulsterones have been reported to lower lipids and cholesterol levels [4–6], to be useful in the treatment of various cardiovascular diseases [7], and to be endowed with anti-neoplastic properties [8].

A number of studies have shown that the biological activity of guggulsterones is at least partly due to their action as ligands of the farnesoid X receptor (FXR) [9–11]. Both isomers **1** and **2** were found to selectively modulate FXR gene expression and, in particular, to positively regulate the expression of the cytochrome Cyp7A1, thus inducing the cholesterol catabolism in bile acids and lowering cholesterol levels. Following experimental evidences, however, indicated that additional pathways are involved in the pharmacological action of guggulsterones. In fact, they may exert their biological effects by the modulation of transcriptor factors as nuclear factor kappa B (NFκB), STAT-3 and C/EBP alpha [12–14], as well as of endocrine steroid and metabolic lipid receptors [15,16].

On the basis of these considerations, there is a strong demand for guggulsterones to better define their biological mechanisms and clinical significance. Being not easily available by extraction procedures (yield: 1.1%) because of their low content in the gum resin, the availability of guggulsterones **1** and **2** comes mainly from synthetic preparations. The first synthesis was reported by Benn and Dodson in 1964 [17], even before their isolation from guggulipids [18]. The method involves the use of 16-dehydropregnenolone acetate (**3**, 16-DIPA) (Fig. 1) as starting material, which is not commercially available and needs to be synthesized besides the low overall yields. In the course of recent years, a number of patents claims improved protocols for the preparation of isomeric mixture of guggulsterones [19,20], while very recently, a regioselective synthesis of *E*-guggulsterone (**1**) has been described from 16,17-epoxy-pregnenolone (**4**) (Fig. 1) via hydrazine reduction and Oppenhauer oxidation [21].

As a part of our ongoing research program in the field of FXR modulators [22] and in the synthesis of natural bioactive steroids [23], herein we report a new, efficient and gram scale regioselective synthesis of *E*-guggulsterone as well as the description of a valuable HPLC protocol for the chromatographic evaluation of both isomers **1** and **2**. At this regard, attempts to obtain the *Z*-isomer **2** from **1** are also described.

Abbreviations: GS, guggulsterone; FXR, farnesoid X receptor.

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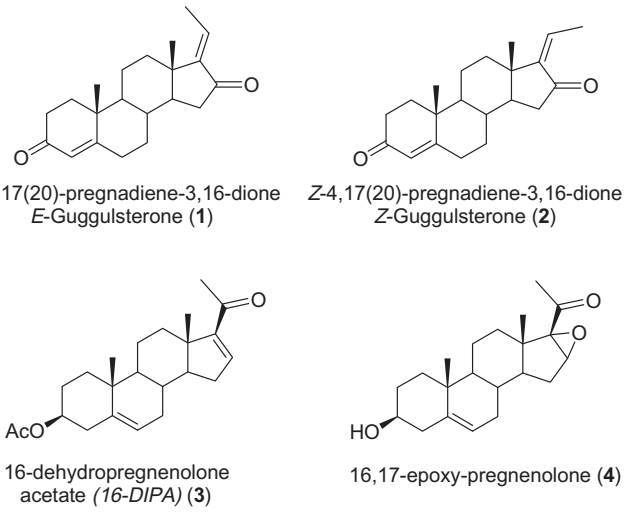

Fig. 1. Structure of *E*- and *Z*-guggulsterone, and related synthetic precursors.

2. Experimental

2.1. Materials

All reagents were commercially available unless otherwise noted. The final products were purified by chromatography on silica-gel (70–230 mesh). TLC was performed on aluminum backed silica plates (silica gel 60 F_{254}). Spots on TLC were visualized by using UV and by staining and warming with phosphomolybdate reagent (5% solution in EtOH). All the reactions were performed using distilled solvents. ^{1}H NMR spectra were recorded at 200 and 400 MHz, ^{13}C NMR spectra were recorded at 100.6 MHz, using the solvents indicated below. Chemical shifts are reported in parts per million (ppm). The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; psd, pseudo singlet. Melting points were determined with an electrothermal apparatus and are uncorrected. Optical rotations were measured with a Jasco Dip-1000 polarimeter in $CHCl_3$.

2.2. Synthesis

2.2.1. 3-Ethoxyandrosta-3,5-dien-17-one (**6**)

Triethyl orthoformate (2.60 mL, 15.63 mmol) and *p*-toluensulfonic acid (0.027 g, 0.14 mmol) were added to a stirred solution of androsten-3,17-dione (**5**) (2.00 g, 6.98 mmol) in freshly distilled THF (20 mL) and absolute EtOH (0.64 mL) under N_2 atmosphere. The resulting mixture was heated at 45 °C for 2 h. The reaction was quenched with 10% $NaHCO_3$ (5 mL) and extracted with Et_2O (3 × 15 mL). The collected organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure, to give **6** in almost quantitative yield (2.19 g, 6.97 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.90 (s, 3H, 18-CH_3), 0.99 (s, 3H, 19-CH_3), 1.24–1.31 (m, 3H, OCH_2CH_3), 3.73–3.81 (m, 2H, OCH_2CH_3), 5.11 (s, 1H, 4-C*H*), 5.21 (s, 1H, 6-C*H*). ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ: 13.6, 14.6, 18.9, 20.4, 21.8, 25.4, 30.7, 31.4, 31.5, 33.7, 35.2, 35.8, 47.6, 48.4, 51.91, 62.1, 98.8, 117.0, 141.21, 154.6, 221.0.

2.2.2. (17Z)-pregna-4,17-dien-3-one (**8**)

Potassium *t*-butoxide (3.02 g, 26.90 mmol) was added to a stirred solution of ethyl-triphenylphosphonium bromide (6.56 g, 17.62 mmol) in distilled THF (50 mL) and under N_2 atmosphere. The resulting suspension was reacted at 60 °C for 90 min. A solution of **6** (1.09 g, 3.49 mmol) in THF (25 mL) was then added dropwise and reacted at 60 °C for 18 h. The reaction mixture was cooled at room temperature, diluted with H_2O (30 mL) and extracted with Et_2O (3 × 25 mL). The collected organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was dissolved in THF (50 mL) and treated with HCl 37% (1 mL) at room temperature for 2 h. The mixture was diluted with EtOAc (30 mL), washed with 10% $NaHCO_3$ (2 × 20 mL), brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography with petroleum ether/EtOAc to give **8** in 95% yield (0.98 g, 16.73 mmol). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 0.91 (s, 3H, 18-CH_3), 1.18 (s, 3H, 19-CH_3), 1.63 (d, 3H, J = 7.1 Hz, 21-CH_3), 5.07–5.18 (m, 1H, 20-C*H*), 5.72 (s, 1H, 4-C*H*). ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ: 13.0, 16.7, 17.3, 20.9, 24.1, 31.2, 31.8, 32.8, 33.9, 35.1, 35.6, 36.8, 38.6, 44.0, 53.7, 55.5, 113.6, 123.7, 149.5, 171.4, 199.5.

2.2.3. (17E)-16α-hydroxy-pregna-4,17-dien-3-one (**9**)

t-Butylhydroperoxide (TBHP) (0.16 mL, 0.88 mmol) was added to a stirred suspension of SeO_2 (0.041 g, 0.37 mmol) in distilled CH_2Cl_2 (3 mL) under N_2 atmosphere at 0 °C. The resulting mixture was reacted for 30 min. A solution of **8** (0.022 g, 0.74 mmol) in CH_2Cl_2 (3 mL) was then added dropwise, warmed to room temperature and reacted at this temperature overnight. The reaction was quenched with $NaHCO_3$ 10% (5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The collected organic layers were washed with brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography with petroleum ether/EtOAc to give 0.208 g (0.66 mmol, 90%) of **9**. mp: 132–134 °C. $C_{21}H_{30}O_2$: Calcd. C, 80.21; H, 9.62. Found. C, 80.30; H, 9.30. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.89 (s, 3H, 18-CH_3), 1.16 (s, 3H, 19-CH_3), 1.71 (d, 3H, J = 7.2 Hz, 21-CH_3), 4.42 (br d, 1H, J = 4.0 Hz, 16-C*H*), 5.57 (q, 1H, J = 7.2 Hz, 20-C*H*), 5.71 (s, 1H, 4-C*H*). ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ: 13.2, 17.2, 17.3, 21.0, 31.7, 32.7, 33.9, 34.4, 34.9, 35.5, 36.9, 38.5, 44.1, 51.8, 53.7, 74.1, 119.8, 123.8, 154.6, 171.1, 199.5.

2.2.4. E-guggulsterone (**1**)

Oxalyl chloride (0.064 mL, 0.74 mmol) was added to a stirred solution of DMSO (0.11 mL, 1.49 mmol) in distilled CH_2Cl_2 (3 mL) under N_2 atmosphere at −50 °C. After 10 min a solution of **9** (195 mg, 0.62 mmol) in CH_2Cl_2 (3 mL) was added dropwise and the mixture was reacted at −50 °C for 40 min. Then Et_3N (0.43 mL, 3.10 mmol) was added, and the resulting reaction was stirred at −50 °C for 40 min, at −20 °C for additional 2 h, and finally warmed at room temperature. The mixture was diluted with CH_2Cl_2 (10 mL) and treated with HCl 3 N (10 mL) for 10 min. The organic phase was separated, washed with H_2O (5 mL), $NaHCO_3$ 10% (5 mL), brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography with petroleum ether/EtOAc to give the desired *E*-guggulsterone (**1**) (0.165 g, 0.52 mmol, 85%). R_f (petroleum ether/EtOAc-7:3, v/v) 0.18. mp: 169–171 °C. $C_{21}H_{28}O_2$: Calcd. C, 80.73; H, 9.03. Found. C, 79.89; H, 8.88. $[\alpha]_D^{20}$ −27.4° (c = 0.016, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.08 (s, 3H, 18-CH_3), 1.25 (s, 3H, 19-CH_3), 1.86 (d, 3H, J = 7.3 Hz, 21-CH_3), 5.75 (s, 1H, 4-C*H*), 6.52 (q, 1H, J = 7.5 Hz, 20-C*H*). ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ: 13.1, 17.3, 17.5, 20.6, 31.8, 32.5, 33.8, 34.2, 35.4, 35.9, 37.7, 38.6, 43.0, 49.5, 53.3, 124.1, 129.5, 147.4, 170.1, 199.2, 205.6.

2.2.5. General procedure for the synthesis of Z-guggulsterone (**2**)

E-guggulsterone (**1**) (0.16 mmol) was dissolved in the appropriate solvent (10 mL) and treated with the catalyst (0.3 equivalents). The mixture was stirred at room temperature for 7 h. The reaction mixture was filtered (when needed), washed with 10% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated under