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Short communication

SHORT COMMUNICATION

Synthesis, crystal structure and local anti-inflammatory activity of the L-phenylalanine methyl ester derivative of dexamethasone-derived cortienic acid

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Abstract: The L-phenylalanine methyl ester derivative of dexamethasone-derived cortienic acid (DF) was synthesized and its crystal structure characterized by the X-ray diffraction method. The crystal system is orthorhombic with space group $P2_12_12_1$ and cell constants a = 8.2969(3) Å, b = 18.9358(8) Å, c = 20.0904(6) Å, V = 3156.4(2) Å^3 and Z = 4. Ring A of the steroid nucleus and phenyl ring in the 17β-side chain are almost planar. Rings B and C have a slightly distorted chair conformation, whereas ring D has an envelope conformation. The packing of DF is characterized by a network of intermolecular hydrogen bonds involving the O4 atom from one side of the steroid nucleus and O1 and F1 atoms from the other side as hydrogen bond acceptors. Apart from the intermolecular hydrogen bonds in the crystal packing, there are also numerous intramolecular hydrogen bonds of the N–H···O, C–H···O and C–H···F type. The local anti-inflammatory activity of DF was evaluated using the croton oil-induced ear oedema test. This derivative achieved maximal inhibition of ear oedema at significantly lower concentration in comparison with dexamethasone.

Keywords: 17β-carboxamide steroids; X-ray diffraction; biological activity; ear oedema test.

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INTRODUCTION

Soft glucocorticoids are compounds synthesized using the retrometabolic approach and usually administered locally near the site of action. After local administration, these derivatives are easily biotransformed to non-toxic and inactive metabolites, resulting in fewer side effects than traditional glucocorticoids. The first (loteprednol etabonate) and second (etiprednol dicloacetate) generations of soft glucocorticoids are derived from cortienic acid (an inactive glucocorticoid metabolite).[1–3] Generally, other derivatives of glucocorticoids that are easily metabolized after local administration to non-toxic and inactive metabolites could also be denoted as soft (antedrug) glucocorticoids.[4] Several groups of 7β-carboxamide steroids were synthesized and tested for glucocorticoid activity. Some of these compounds showed significant glucocorticoid activity (inhibition of phytohaemagglutinin-induced blastogenesis of lymphocytes).[5–7] However, their metabolic properties and toxicity have not been tested so far.

A novel class of 17β-carboxamide derivatives of glucocorticoids was recently presented. These derivatives are amides of cortienic acids obtained from hydrocortisone, prednisolone, methylprednisolone, dexamethasone and betamethasone with amino acids. Molecular docking calculations indicate that introduction of an amino acid moiety in the 17β side chain enables favourable orientation in the glucocorticoid receptor (GR) and key binding interactions with the amino acids from the GR.[8] Their permeability and retention in human skin were predicted by use of *in vitro* tests – parallel artificial membrane permeability assay (PAMPA) and biopartitioning micellar chromatography.[9,10] According to these results, L-phenylalanine methyl ester derivatives should have significant local anti-inflammatory activity and a better skin retention/permeability ratio in comparison with corresponding parent glucocorticoids.

The aim of this study was to synthesize the L-phenylalanine methyl ester derivative of dexamethasone-derived cortienic acid (DF), perform crystallographic analysis of its structure and test the local anti-inflammatory activity of this derivative.

EXPERIMENTAL

Materials and methods

Dexamethasone was purchased from Tokyo Chemical Industry (Tokyo, Japan), whereas *N*-hydroxybenzotriazole (HOBt), *N*,*N*′-dicyclohexylcarbodiimide (DCC), croton oil, acetonitrile, *N*,*N*-dimethylformamide (DMF) and silica gel for preparative thin-layer chromatography were purchased from Sigma–Aldrich (Steinheim, Germany). Triethylamine (TEA) and L-phenylalanine methyl ester hydrochloride were purchased from Acros Organics (Geel, Belgium), chloroform and methanol from JT Baker (Loughborough, UK) and acetone from Zorka (Šabac, Serbia). Silica gel for column chromatography was purchased from Merck (Darmstadt, Germany).

Apparatus

The melting point was determined using a Boetius PHMK 05 apparatus (Radebeul, Germany). The UV spectrum was recorded on an Evolution 300 spectrophotometer (Thermo Scientific, UK), whereas the IR spectrum was recorded using a Nicolet iS10 ATR-FTIR spectrophotometer (Thermo Scientific, Madison, WI, USA). The ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker Avance III 400 NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany), operating at 400 MHz for protons and 100 MHz for carbons. The accurate mass was determined using an Agilent 6210 time-of-flight mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The crystallographic data were collected on an Oxford Diffraction Gemini S diffractometer.

Synthesis

The precursor (dexamethasone-derived cortienic acid, CD) was synthesized by periodic acid oxidation of dexamethasone, following a reported method.[9]

DF was synthesized from CD and L-phenylalanine methyl ester hydrochloride by use of DCC, HOBt and TEA according to a reported procedure,[8] which was a modification of a previously published method.[11] CD (53 mg, 0.14 mmol, 1 eq) was dissolved in DMF (2 mL) and the solution was cooled to 0 °C. Subsequently, DCC (58 mg, 0.28 mmol, 2 eq) and HOBt (28 mg, 0.21 mmol, 1.5 eq) were added. The mixture was stirred at 0 °C for 1 h and thereafter maintained at a temperature not exceeding 8 °C for 15 h. L-Phenylalanine methyl ester hydrochloride (30 mg, 0.14 mmol, 1 eq) was dissolved in DMF (1 mL), TEA was added (39 μL, 2 eq) and the mixture was cooled to 0 °C. Finally, the mixture of CD, DCC and HOBt was filtered and added dropwise. This reaction mixture was stirred at 0 °C for 1 h and maintained at a temperature not exceeding 8 °C for 15 h. The reaction mixture was filtered and evaporated to dryness under reduced pressure. Column chromatography was employed for the initial purification of the reaction mixture, whereas the final purification was realised by preparative thin-layer chromatography. Mobile phase used for column chromatography purification was chloroform/methanol 99:1 (*V*/*V*), whereas the mobile phase used for the purification by preparative thin-layer chromatography was chloroform/methanol 95:5 (*V*/*V*). The purified compound was recrystallized from water/acetonitrile 50:50 (*V*/*V*). Yield: 83.5 %.

X-Ray crystallography

Details of crystal data, data collection and structure refinement are summarized in Table S-I of the Supplementary material to this paper.

The structure was solved by the direct method using the program SHELXS-97[12] and refined by SHELXL-97.[13] The H atoms bonded to the N and O atoms were located from difference Fourier maps and the H atoms bonded to C atoms were placed at the geometrically calculated positions and refined using a riding model. C–H distances were fixed at 0.93 Å for aromatic C atoms, 0.97 Å for secondary C–H2 groups, 0.98 Å for tertiary C–H groups and 0.96 Å for methyl C–H3 groups. Their U_{iso}(H) values are equal to 1.2U_{eq} of the corresponding C atom, except for the methyl groups where the U_{iso}(H) values were set to 1.5U_{eq}(C). In the absence of significant anomalous scattering, the absolute configuration could not be reliably determined and any reference to the Flack parameter[14] was removed. In the phenyl ring, atoms C27, C28 and C29 show slightly elongated atomic displacement ellipsoids. Attempts to model disorder for the rings, even by employing extensive restraints, proved fruitless. Examination of the refined structure using PLATON[15] revealed a total void volume of 280 Å^3 distributed over two sites (–0.060, 0.250, 0.500) and (0.031, 0.750, 0.000).

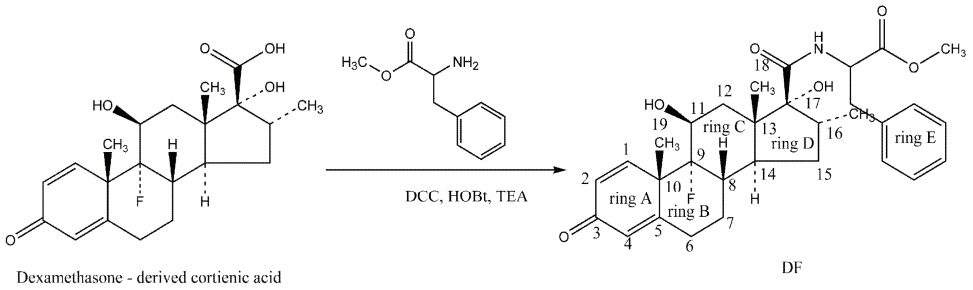
Local anti-inflammatory activity assay

The local anti-inflammatory activities of DF and dexamethasone were evaluated using the croton oil-induced ear oedema test,[16-19] following the procedure described elsewhere.[8] The experimental procedure was in accordance with the institutional guidelines for care and use of animals in research No 03/10-2013 (Ethics Committee in research of the Military Medical Academy, Belgrade, Serbia). Croton oil solution (35 μg mL^{-1}), five solutions of DF (1.15, 2.29, 4.58, 9.17 and 13.75 μM) and five solutions of dexamethasone (9.17, 13.75, 27.50, 36.70 and 45.80 μM) were prepared in acetone. Eleven groups of experimental animals were formed – a control group and ten test groups (five groups for DF and five groups for dexamethasone, each containing five rats).

RESULTS AND DISCUSSION

Synthesis and physicochemical characterization

DF was previously synthesized using a single-step procedure, which utilizes 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC), HOBt and TEA.[9] In this paper, an alternative two-step synthesis of DF is presented (Scheme 1). By use of the two-step procedure, DF was synthesized in good yield (83.5 %), which is significantly higher in comparison with that obtained using the single-step synthesis (52.1 %).



Scheme 1. Synthesis of DF.

DF was characterized by determining the melting point as well as by use of spectroscopy techniques (UV, IR, NMR, MS/MS and MS-TOF spectroscopy). The physical, analytical and spectral data for the title compound are given in the Supplementary material to this paper.

Crystal structure

DF crystallizes in the space group $P2_12_12_1$. Its molecular structure with the atom-labelling scheme is shown in Fig. 1. Selected bond lengths and bond angles are listed in Table S-II of the Supplementary material to this paper.

There are four fused rings, one five-membered (D) and three six-membered (A, B and C) rings. The five-membered ring D has an envelope conformation, with atom C13 at the flap position displaced by 0.757(2) Å from the best plane of the other four C atoms of the D ring ($Q(2)$ = 0.5038(17) Å, $\varphi(2)$ = 177.6(2)°).

The cyclohexane rings B and C have a slightly distorted chair conformation. The key puckering parameter[20] for a chair conformation should be $\theta = 0°$ for an ideal chair. The values of θ are 10.16(18) and 9.26(18)° for rings B and C, respectively. Other puckering parameters for ring B are Q = 0.5607(18) Å and φ = = 283.6(10)°, whereas for ring C, they are Q = 0.5311(17) Å and φ = 271.9(11)°. Rings A (C1–C2–C3–C4–C5–C10) and E (C24–C25–C26–C27–C28–C29) are almost planar with an average atom displacement of 0.015 Å for C24–C25–C26––C27–C28–C29 and 0.010 Å for C1–C2–C3–C4–C5–C10 from the plane defined by all atoms of the cyclohexane ring.

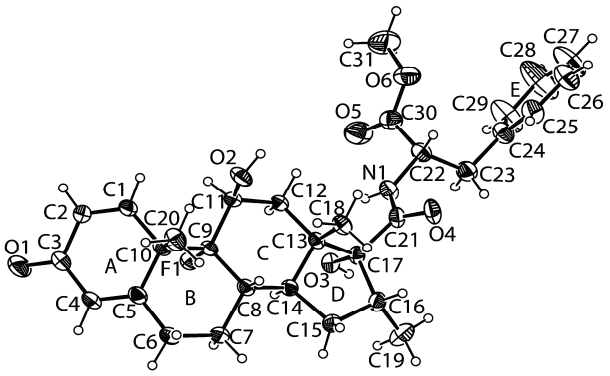


Fig. 1. Molecular structure of DF showing the atom-labelling scheme. Displacement ellipsoids are drawn at the 30 % probability level. The H atoms are shown as small circles of arbitrary radii.

The bonds C10–C20 and C9–F1 adopt the axial position at 2.95(10) and 8.89(9)°, respectively, with respect to ring B of the steroid nucleus, whereas bond C16–C19 occupies a bisectional position at 56.1(1)° with respect to ring D. Bonds C13–C18 and C11–O2 adopt axial positions at 5.43(9) and 15.50(10)°, respectively, with respect to the ring C of the steroid nucleus.

The length of the steroid nucleus C3···C16 is 8.586 Å and the dihedral angle between the mean planes of the steroid nucleus and the phenyl moiety (ring E) is 78.73(12)°.

The packing of DF is characterized by a network of hydrogen bonds involving the O4 atom from one side of the steroid nucleus and the O1 and F1 atoms from the other side as hydrogen bond acceptors. In the crystallographic *b* direction, chain growth is ensured by means of the O3–H···O1 and C29–H···F1 interactions, forming a zigzag pattern (Fig. 2a and Table S-III of the Supplementary material). Similar zigzag pattern is formed down the crystallographic *a*-axis by O2–H···O4 hydrogen bonds (Fig. 2b and Table S-III). Besides the intermolecular hydrogen bonds in the crystal packing, there are numerous intramolecular hydrogen bonds of the N–H···O, C–H···O and C–H···F type (Table S-III).

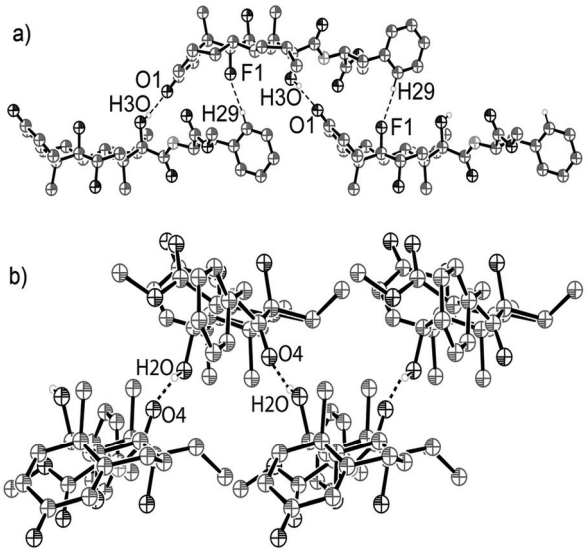


Fig. 2. The crystal packing of DF viewed down: a) [100] showing the O3–H3O…O1 and C29––H29…F1 hydrogen bonds, and b) [010] showing the O2––H2O…O4 hydrogen bond. The hydrogen bonds are shown as dotted lines. For clarity H atoms not participating in the interactions are omitted.

Local anti-inflammatory activity

Maximal inhibition of ear oedema caused by DF (29.54 %) was lower than the maximal inhibition caused by dexamethasone (55.54 %). However, DF could be applied at a lower concentration, because this derivative caused maximal inhibition of ear oedema at a significantly lower concentration (4.58 μM) in comparison with dexamethasone (45.8 μM). Additionally, DF should have significantly better skin retention/permeability ratio.[9] The local anti-inflammatory profile of DF is presented in Fig. 3.

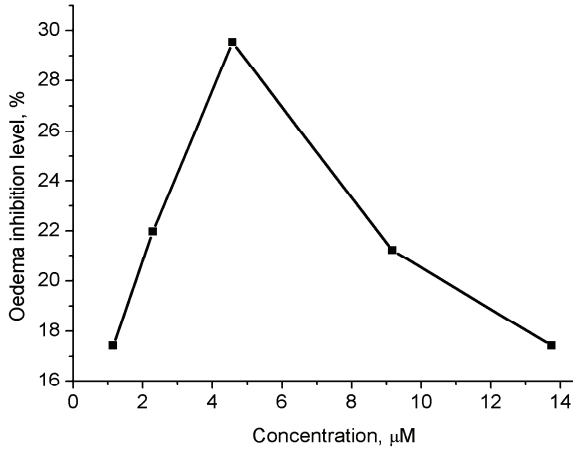


Fig. 3. Local anti-inflammatory profile of DF.

CONCLUSIONS

The L-phenylalanine methyl ester derivative of dexamethasone-derived cortienic acid (DF) was synthesized and structurally characterized. This derivative is a potential soft drug with fewer side effects and a better skin retention/permeability ratio than dexamethasone. Its crystal structure was characterized by the X-ray diffraction method. Ring A of the steroid nucleus and the phenyl ring (17β-side chain) are almost planar, rings B and C have a slightly distorted chair conformation and ring D has an envelope conformation. Hydrogen bonds that influence crystal packing of this derivative were identified, *i.e.*, O2–H···O4 (crystallographic *a* direction), and O3–H···O1 and C29–H···F1 interactions (crystallographic *b* direction). The local anti-inflammatory activity of DF was evaluated by the croton oil-induced ear oedema test. This derivative possesses local anti-inflammatory activity with the maximal inhibition of ear oedema achieved at significantly lower concentration in comparison with dexamethasone.

SUPPLEMENTARY MATERIAL

The physical, spectral and crystal data for DF, selected bond lengths and angles, as well as hydrogen bond geometry of DF are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC-1034681. Copies of these can be obtained free of charge on written application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033); on request by e-mail to deposit@ccdc.cam.ac.uk or by access to http://www.ccdc.cam.ac.uk.

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ИЗВОД

СИНТЕЗА, КРИСТАЛНА СТРУКТУРА И ЛОКАЛНА АНТИИНФЛАМАТОРНА АКТИВНОСТ ДЕРИВАТА КОРТИЕНСКЕ КИСЕЛИНЕ ИЗ ДЕКСАМЕТАЗОНА И МЕТИЛ-ЕСТРА L-ФЕНИЛАЛАНИНА

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Синтетисан је дериват кортиенске киселине из дексаметазона и метил-естра L-фенилаланина (DF), а кристална структура овог једињења окарактерисана је методом дифракције X-зрака. Кристални систем је орторомбичан, са просторном групом $P2_12_12_1$ и константама a = 8,2969(3) Å^3, b = 18,9358(8) Å^3, c = 20,0904(6) Å^3, V = 3156,4 Å^3 и

Z = 4. Прстен A стероидне структуре и бензенов прстен у 17β-бочном низу су скоро планарни. Прстенови B и C су у благо искривљеној конформацији столице, док је прстен D у конформацији коверте. Кристална структура DF се карактерише мрежом интермолекулских водоничних веза преко којих се атом O4 са једне стране стероидне структуре повезује са атомима O1 и F1 (акцептори водоничне везе) са друге стране стероидне структуре. Поред интермолекулских, присутне су и бројне интрамолекулске водоничне везе N–H···O, C–H···O и C–H···F типа. Локална антиинфламаторна активност DF је испитана применом теста инхибиције едема уха изазваног кротонским уљем. Овај дериват постиже максималну инхибицију едема уха при значајно нижој концентрацији у односу на дексаметазон.

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