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SYNTHESIS AND ANTIOXIDANT EVALUATION OF SOME NEW ALLOBETULIN ESTERS

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ABSTRACT

A series of 3-O-haloacyl allobetulin were synthesized via acylation of allobetulin by haloacetic (trifluro,difluorochloro, trichloro, monochloro) acid in $CHCl_3$ at 70 °C. The structure of allobetulin was modified at C-3 position, and esters **3a-d** were obtained in high yields. Moreover the reactions do not need a catalyst. The structures of all synthesized compounds were confirmed by various spectroscopic methods (IR and NMR) and tested for antioxidant activity.

Keywords: triterpene, betulin, allobetulin, esterification, haloacetic acid, antioxidant.

INTRODUCTION

Triterpenes are compounds derived from the natural precursor, squalene. After the cyclization of epoxidized squalene (oxidosqualene) numerous polycyclic triterpenic structures are formed, mostly with the C-3 hydroxyl group, one or more carboxyl and additional hydroxyl groups may present as well. The presence of hydroxyl functional group in such a polycyclic structure makes the possibility to conduct various chemical transformations leading to numerous new derivatives.

Recently, various interesting biological activities were found for this class of compounds, which in combination with their low toxicities prompt an increased research effort [1,2]. More remarkably, the oleanane group that exhibits a number of relevant pharmacological activities.

Allobetulin (19β,28-epoxyoleanan-3-ol) **1**, the oleanane series terpenoid, is widely represented in the plant world. In particular, it is a part of the extractive substances of birch bark, the main component of which is betulin, the lupane series triterpenoid. Synthetic allobetulin has been easily obtained from betulin by acid-catalyzed intramolecular cyclization, which involves the hydroxymethyl group CH_2OH of C-28 and C_{29}=C_{20} double bond, accompanied by Wagner–Meerwein type rearrangement.[3]

Allobetulin, is the excellent example of chemical reactivity of C-3 hydroxyl group and the esterification of this latter leading to the receiving of diverse derivatives with significant pharmacological properties.

The syntheses of different ester derivatives of allobetulin have been described comprehensively in the literature *via* acylation of the 3-βOH function.[4-6]

Allobetulin and its derivatives bearing an ester group at C-3 show a wide range of biological activity. They have an anti-inflammatory activity comparable to ortophen (diclofenac) [6,7], antiulcer activity [7,8], antiviral activity [9], antifeedant and insecticidal activities.[10]

An analysis of published data suggests that allobetulin derivatives containing acyl groups are a promising source of new biologically active substances. We report herein the synthesis of a series of some new halogen-containing esters of allobetulin, and evaluation of their antioxidant activities.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra have been recorded via Bruker AVANCE 400 III HD spectrometer (Bruker, Billerica, MA, USA), 400.17 and 100.63 MHz, respectively. Chemical shifts (d) are reported relative to tetramethylsilane peak set at 0.00 ppm. In the case of multiplets, the signals are reported as intervals.

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Signals were abbreviated as s, singlet; d, doublet; t, triplet; m, multiplet. Coupling constants were expressed in Hz.
TLC was conducted on Sorbfil plates using C_6H_6:CH_2Cl_2:CH_3OH (5:5:1). Spots were detected by spraying TLC plates with 1% phosphomolybdic acid and heating at 110 ºC for 5 minutes to show a characteristic blue color.
Infrared spectra were obtained directly from the products using Bruker Tensor 27 FT-IR Spectrometer. The spectra were recorded in the range of 400 to 4000 cm^{-1}. Melting temperatures have been detected in open capillaries using Buchi apparatus.

Voltammetric Approach for Antioxidant Activity Assessment
In this work, we applied the voltammetric technic for the measurement of antioxidant properties. This method was previously used for antioxidant activity determination of pharmaceutical substrate and biological objects [11, 12].
The voltammetric analyzer TA-2 (Tomanalyt, Tomsk, Russia) was used to record cathode current. We used a glass cup with a background electrolyte solution of 10 cm3 in volume as an electrochemical cell. A three-electrode circuit with an indicator mercury film electrode, a silver-silver chloride reference electrode and silver-silver chloride auxiliary electrode was used with 0.1 M NaClO4 in ethanol (96% ethyl alcohol) as a background solution.
The change of current ER O_2 was recalculated to the activity of the test substance. The coefficient of antioxidant activity was expressed in $\mu mol \cdot l^{-1} min^{-1}$, which reflects the amount of active oxygen radicals interacted with the antioxidants in a time period, as follows:

$$K = \frac{C^0}{t}\left(1 - \frac{I}{Io}\right)$$

Where C^0 [$\mu mol \cdot l^{-1}$] is the oxygen concentration in solution, I is the ER O_2 current with the investigated substance addition in the solution, I_o is the limiting ER O_2 current without the substance in the solution, t (min) is the time of the interaction between the reactive oxygen species and an antioxidant at the working electrode.
Samples were examined at a final concentration of 0.0125% (0.125 mg/ml). The sample of a mass 0.0100 g was taken and dissolved in 1 ml of DMF and then 3 ml of 96% ethanol was added. The aliquot of 0.5 ml was taken from the resulting solution and placed into the electrochemical cell. Three parallel tests were made, the average coefficient of antioxidant activity was calculated. The results were statistically processed.

General Procedure
To a solution of betulin (1.0 equivalent) in $CHCl_3$ 20 ml was added haloacetic acid (2.5 equivalents) and stirred at 70 °C for 2-6 hours. After completion of the reaction (as indicated by TLC), the filtrate was evaporated and the methanol was added to form a precipitate, which was separated by filtration. The white residue was recrystallized from acetone.

Allobetulin 3-O- monochloroacetate (3a)
yield 96 %, Rf= 0.56, mp=248 °C. IR spectrum (KBr, ν, cm–1): 2926.7–2866.2 (-CH_3 and -CH_2), 1749.4 (C=O), 1292.0 and 1031.1 (C-O-C), 799.5 (C-Cl). ^{1}H NMR spectrum (400.17 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.70 (3 H, s), 0.74 (3 H, s), 0.80 (3 H, s), 0.81 (3 H, s), 0.85 (3 H, s), 0.86 (3 H, s), 0.91 (3 H, s), 4.03 (2 H, s, CH_2CO), 3.38 (1 H, d, *J*= 7.6, 28-Ha), 3.50 (1 H, s, 19a-H), 3.71 (1 H, d, *J*= 7.6, 28-Hb), 4.50 (1 H, m, 3a-H). ^{13}C NMR spectrum (100.63 MHz, $CDCl_3$, δ, ppm): 12.47 (C27), 14.66 (C24), 15.42 (C26), 15.52 (C25), 17.06 (C6), 19.99 (C11), 22.52 (Cl**CH$_2$**COO), 23.51 (C29 or C30), 25.17 (CH_2), 25.36 (CH_2), 26.88 (CH_2), 27.75 (C23), 31.65 (C29 or C30), 32.77 (C21), 33.06 (C7), 35.20 (C13), 35.65 (C17), 36.12 (C16), 37.00 (C10), 37.48 (C4), 39.59 (C1),39.69 (C), 40.44 (C), 45.75 (C18), 49.94 (C9), 54.48 (C5), 70.15 (C28), 82.36 (C3), 87.03 (C19), 166.19 (ClCH$_2$**C**OO).

Allobetulin 3-O- difluorochloroacetate (3b)
yield 98 %, Rf= 0.57, mp=258 °C. IR spectrum (KBr, ν, cm–1): 2943.6–2881.2 (-CH_3 and -CH_2), 1765.4 (C=O), 1169.7 and 1033.4 (C-O-C), 732.6 and 834.6 (C-Cl), 1136.2 (C-F). ^{1}H NMR spectrum (400.17 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.81 (3 H, s), 0.91 (3 H, s), 0.92 (3 H, s), 0.93 (3 H, s), 0.94 (3 H, s), 0.96 (3 H, s), 1.01 (3 H, s), 3.48 (1 H, d, *J*= 7.6, 28-Ha), 3.56 (1 H, s, 19a-H), 3.79 (1 H, d, *J*= 7.6, 28-Hb), 4.69 (1 H, m, 3a-H). ^{13}C NMR spectrum (100.63 MHz, $CDCl_3$, δ, ppm): 13.50 (C27), 15.70 (C24), 16.26 (C26), 16.53 (C25), 18.05 (C6), 21.04 (C11), 24.55 (C29 or C30), 26.23 (CH_2), 26.37 (CH_2), 26.42 (CH_2), 27.40 (C2), 27.81 (C23), 28.80 (C29 or C30), 32.69 (C21), 33.77 (C7), 34.11 (C13), 36.27 (C17), 36.71 (C16), 37.16 (C10), 38.24 (C4), 38.42 (C1), 40.63 (C), 40.74 (C), 41.48 (C), 46.80 (C18), 50.97 (C9), 55.47 (C5), 72.35 (C28), 86.43 (C3), 88.00 (C19),117.05 (**C**ClF_2COO), 159.13 ($CClF_2$**C**OO).

Allobetulin 3-O- trifluoroacetate (3c)
yield 99 %, Rf= 0.54, mp=268 °C. IR spectrum (KBr, ν, cm–1): 2944.6–2869.0 (-CH_3 and -CH_2), 1770.1 (C=O), 1219.3, 1188.6 and 1033.0 (C-O-C). 1166.6 (C-F). ^{1}H NMR spectrum (400.17 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.72 (3 H, s), 0.81 (3 H, s), 0.82 (3 H, s), 0.84 (3 H, s), 0.85 (3 H, s), 0.87 (3 H, s), 0.91 (3 H, s), 3.37 (1 H, d, *J*= 7.6, 28-Ha), 3.47 (1 H, s, 19a-H), 3.70 (1 H, d, *J*= 7.6, 28-Hb), 4.63 (1 H, m, 3a-H). ^{13}C NMR spectrum (100.63 MHz, $CDCl_3$, δ, ppm): 12.46 (C27), 14.73 (C24), 15.22 (C26), 15.49 (C25), 17.04 (C6), 20.01 (C11),22.23 (CH_2), 23.52 (C29 or C30), 25.49 (CH_2), 26.73 (CH_2), 26.84 (C2), 27.77 (C23), 31.66 (C29 or C30), 32.74 (C21), 33.09 (C7), 35.24 (C13), 35.69 (C17), 36.12 (C16), 37.05 (C10), 37.39 (C4), 39.60 (C1), 39.71 (C), 40.44 (C), 45.77 (C18), 49.94 (C9), 54.40 (C5), 70.22 (C28), 85.25 (C3), 86.93 (C19). 115.09 (**C**F_3COO), 156.57 (CF_3**C**OO).

Allobetulin 3-O- trichloroacetate (3d)
yield 96 %, Rf= 0.55, mp=285 °C. IR spectrum (KBr, ν, cm–1): 2946.1–2856.5 (-CH_3 and -CH_2), 1763.5 (C=O), 1282.9 and 1033.7 (C-O-C), 799.5 (C-Cl). ^{1}H NMR spectrum (400.17 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.81 (3 H, s), 0.91 (3 H, s), 0.93 (3 H, s), 0.94 (3 H, s), 0.95 (3 H, s), 0.99 (3 H, s), 1.05 (3 H, s), 3.46 (1 H, d, *J*= 7.6, 28-Ha), 3.57 (1 H, s, 19a-H), 3.79 (1 H, d, *J*= 7.6, 28-Hb), 4.63 (1 H, m, 3a-H). ^{13}C NMR spectrum (100.63 MHz, $CDCl_3$, δ, ppm): 14.25 (C27), 15.70 (C24), 16.33 (C26), 16.53 (C25),18.04 (C6), 21.05 (C11),24.56 (C29 or C30), 26.22 (CH_2), 26.41 (CH_2), 26.44 (CH_2), 26.52 (C2), 27.90 (C23), 28.80 (C29 or C30), 32.69 (C21), 33.78 (C7), 34.11 (C13), 36.25 (C17), 36.70 (C16), 37.11 (C10), 37.80 (C4), 38.44 (C1), 40.64 (C), 40.74 (C), 41.48 (C), 46.79 (C18), 50.95 (C9), 55.43 (C5), 71.23 (C28), 87.36 (C3), 88.03 (C19), 90.53 (**C**Cl_3COO), 161.68 (CCl_3**C**OO).

RESULTS AND DISCUSSION

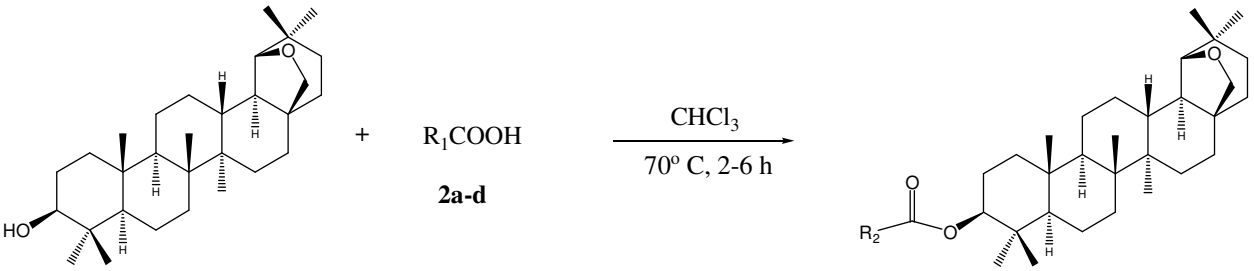
Allobetulin was synthesized directly from Betulin, according to the method described in the literature.[13] The chemical structure of allobetulin was confirmed using ^{1}H and ^{13}C NMR and IR spectroscopy and then compared with the spectral data described in the literature.[14]

Previously, the allobetulin trifluoroacetate has been synthesized from betulin 3-acetate and trifluoroacetic anhydride [9]. However, in the present study, all our esters including allobetulin trifluoroacetate were synthesized from allobetulin directly by treating them with different haloacetic acids.

The synthesis of allobetulin esters is summarized in scheme-1. The structure of allobetulin **1** was modified at the C-3 position. Haloacetic acid **2a-d** were introduced at the C-3 position in dry chloroform ($CHCl_3$) at reflux to obtain compounds **3a-d** in high yields (96-99%) after 2-6 h.

The different reaction times of compounds **3a-d** may be interpreted in terms of various factors. One of these factors is the acidity of haloacetic acid molecules. When a hydrogen atoms at the alpha position of acetic acid are replaced by halogens the acidity of the acetic acid molecule will increase with the addition of each subsequent halogen atom. Furthermore, the acidity of the mono-, di-, and trihalogenated series **2a-d** was enhanced with the increasing electronegativity of the halogen atom in the order F > Cl > Br > I. According to the reported acidities of acetic acid derivatives ClF_2CCOOH pka=0.46 [15], CF_3COOH pka=0.23, $ClCH_2COOH$ pka=2.87, CCl_3COOH pka=0.77 [16]. Their acidity increases in the order **2c > 2b > 2d > 2a**. Thus, an increase in the acidity will increase the electrophilicity of the acid, the higher the

electrophilicity of the acid the faster the reaction time of esterification. The time of reactions increases in the order **3c** (2 h) **> 3b** (2.5 h) **> 3d** (5 h) **> 3a** (6 h)**.**



1 **3a-d**

2a R_1= -CH_2Cl, **2b** R_1= -CF_2Cl, **2c** R_1= -CF_3, **2d** R_1= -CCl_3
3a R_2= -CH_2Cl, **3b** R_2= -CF_2Cl, **3c** R_2= -CF_3, **3d** R_2= -CCl_3

Scheme-1: Synthesis of Allobetulin esters **3a-d**

The composition and structure of adducts **3a-d** were confirmed by IR, ^{1}H NMR and ^{13}C NMR spectra. In their IR spectra, the allobetulin esters have been characterized by the disappearance of OH band of allobetulin and appearance of new bands in the prepared compounds **3a-d** such as the C=O-group at 1730–1775 cm^{-1} and of the C–O ester group at 1000–1275 cm^{-1}.

The bands of the carbon-chlorine bond are observed for chlorine-containing compounds **3a, b** and **d** in low-frequency spectral regions: 670-850 cm^{-1}. The IR spectra of fluorine-containing esters **3b** and **3c** contain intense bands associated with C–F vibrations at 1166.6 and 1136.2 cm^{-1}, respectively.

The ^{1}H NMR spectrum of allobetulin esters synthesized **3a-d** contain characteristic signals of tetrahydrofuran ring, which appears as doublets of protons of CH_2 group (*AB* system), δ 3.45–3.78 ppm, and a singlet of CH group at 3.53–3.57 ppm. Comparison of the ^{1}H NMR spectra of **3a**-**d** and allobetulin revealed an appreciable downfield shift of the C-3 proton signals as a result of introduction of an acyl group (the very characteristic resonance signal from an unsubstituted derivatives is usually at around 3.2–3.3 ppm, while substitution of carbon C-3 shifts this signal downfield by at least 1.0 ppm).

The IR spectrum of the derivative **3a** showed the presence of a new strong pick at 1728 cm^{-1}, characteristic to C=O of ester, and the disappearance of alcohol absorption band between 3200-3600 cm^{-1} was indicative of esterification. In the ^{1}H NMR spectrum the H-3 was shifted downfield from 3.22 ppm in **1** to 4.50 ppm in **3a**, and the same effect was observed in ^{13}C NMR as well from 78.99 ppm in **1** to 82.36 ppm in **3a** which indicates the acylation of allobetulin **1** at C-3. The presence of chloroacetyl side chain $ClCH_2$ was affirmed by the apparition of the characteristic signal at 4.07 ppm as a singlet in ^{1}H NMR and signal at 22.52 ppm in ^{13}C NMR as well. Moreover, the resonance corresponding to the carbonyl ester was shown at 166.19 ppm in the ^{13}C NMR.

The IR spectrum of the derivative **3b** showed a new strong pick at 1732 cm^{-1} attributed to C=O indicating acylation of compound **1** at C-3. Displacement of H-3 signal from 3.22 ppm in **1** to 4.69 ppm in **3b** in ^{1}H NMR and the downfield shift observed at C-3 signal from 78.99 ppm in **1** to 86.43 ppm in **3b** in ^{13}C NMR confirm the presence of an ester-bearing C-3. Side chain was assured by characteristics signals at 117.05 ppm belonging to C-ClF_2 group in the ^{13}C NMR spectrum, besides the new signal at 159.13 ppm assignable to the carbonyl ester.

The presence of carboester groups in the molecule **3c** was confirmed by the IR spectrum which displays the presence of new strong pick at 1736 cm^{-1} attributed to C=O of an ester. The formation of the ester **3c** was also proven by ^{1}H-NMR which indicates downfield displacement of the multiplicity of H-3 from 3.22 ppm in **1** to 4.63 ppm in **3c**. As it was shown in ^{13}C NMR by shift downfield of C-3 signal from 78.99 ppm in **1** to 85.25 ppm in **3c**. In addition, the presence of a signal at 115.09 ppm, which is associated with CF_3 group and the signal belonging to the carbonyl ester that was observed at δ 156.57 ppm have confirmed the achievement of esterification. The appearance of the new pick at 1732 cm^{-1} in the IR spectrum of **3d** confirmed the presence of a carbonyl group in this compound. The 3-O-acylated ester

exhibited multiplicity in ^{1}H NMR with the chemical shift being shifted significantly downfield from 3.22 ppm in **1** to 4.63 ppm in **3d**. As it can be observed in its ^{13}C NMR, which displayed a displacement from 78.99 ppm in **1** to 87.36 ppm **3d**. The change in the chemical shifts at C-3 affirmed the acylation of the hydroxy group. Furthermore, the presence of a signal at 90.53 ppm corresponding to CCl_3 group, and the signal at 161.68 ppm assigned to the carbonyl group (C=O) in the ^{13}C NMR spectrum have confirmed the esterification at C-3.

Pharmacology

Antioxidant Activity

According to the results obtained by voltammetric technic (Fig.-1), all synthesized derivatives **3a-d** exhibited good antioxidant activity in comparison with allobetulin **1**. It should be noted that the activity of these Compounds **3a-d** surpasses the activity of such well-known antioxidant like ascorbic acid 1.165.

The improvement of the antioxidant activity of these compounds may be interpreted by the influence of the alkyl ester side chain. On the comparison between the compounds **3a-d**, it was noticed that the presence of halogen at the alpha position of acetyl group results in an increase in the antioxidant activity. The presence of trihaloacetyl groups significantly enhanced the antioxidant activity of compounds **3b-d** in comparison with compound **3a** with monohaloacetyl group.

Thus, The insertion of an alkyl side chain by using ester as linker has enhanced the antioxidant activity of allobetulin.

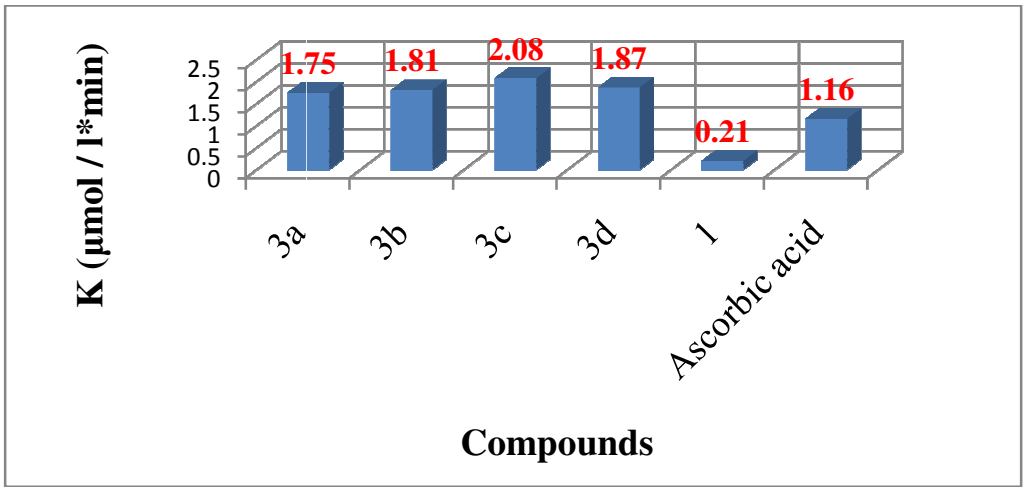


Fig.-1: Antioxidant Activity of Compounds **3a-d**

CONCLUSION

In summary, A series of new ester derivatives of allobetulin has been described by applying a simple esterification reaction without need of a catalyst. All the compounds were obtained with excellent yield. As well as they showed good antioxidant activity in comparison with ascorbic acid.

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