

SULFONATION OF BETULINIC ACID BY SULFAMIC ACID

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Betulinic acid was sulfonated by sulfamic acid in the presence of urea in homogeneous 1,4-dioxane or DMF solution at 65–75°C in 2.5–3.5 h to give betulinic acid 3-sulfate, the structure of which was confirmed by IR and ¹³C NMR spectroscopy.

Keywords: betulinic acid, sulfonation, sulfamic acid, urea, 1,4-dioxane, DMF, betulinic acid 3-sulfate.

Betulinic acid (BA) (**1**) exhibits the most pronounced antitumor activity of lupane triterpenoids found in the bark and leaves of many plants. However, isolation of BA from plant raw material is economically unfeasible because of its low content (up to 0.1%) [1]. Bark of *Betula pendula* Roth. (European white birch) contains up to 35% of betulin, a biosynthetic precursor of BA [2]. It was shown that the antiproliferative activity of BA is an order of magnitude greater than that of the known antineoplastic agent doxorubicin against human melanoma cells [3] and inhibits the growth of cancer cells [4]. A study of the biological activity of BA 3-sulfate showed that it was a stronger complement inhibitor than the currently used medicines [5].

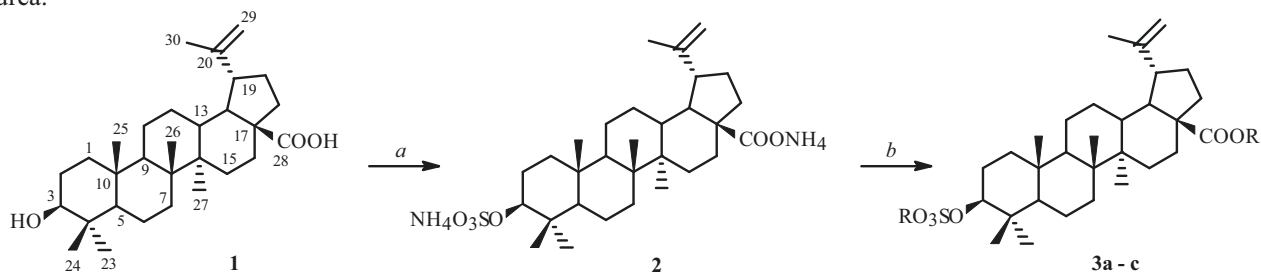
In many instances, the efficacy of medicines depends on their water-solubility.

The broad spectrum of biological activity of BA and its sulfate prompted the development of effective BA sulfonation methods [5–8].

Traditional methods for synthesizing triterpenoid sulfates are based on the use of H₂SO₄, SO₃, and chlorosulfonic acid (ClSO₃H) [6–8]. Sulfamic acid (NH₂SO₃H), in contrast with the aforementioned aggressive reagents, is a stable, nonhygroscopic, crystalline compound that is soluble in DMF and 1,4-dioxane and is just as acidic as H₂SO₄ [8]. Sulfamic acid is produced industrially by the reaction of urea and conc. H₂SO₄. Its reactivity is similar to that of SO₃ complexes with tertiary amines (NR₃·SO₃) [8].

Previous research showed that sulfonation of betulin by NH₂SO₃H in the presence of urea in DMF and 1,4-dioxane formed betulin 3,28-disulfate in 97% yield. The double bond in the isoprenyl group of betulin was unaffected during the reaction with NH₂SO₃H [9]. The catalytic effect of urea during NH₂SO₃H sulfonation was explained by the formation of a donor–acceptor complex that was more reactive toward sulfonation [9, 10]. Sulfonation of BA by NH₂SO₃H has not been reported.

The goal of the present work was to study the reaction of BA and NH₂SO₃H in DMF and 1,4-dioxane in the presence of urea.



R = H (**a**), K (**b**), Na (**c**)

a. NH₂SO₃H, (NH₂)₂CO, DMF or dioxane; *b.* 10% H₂SO₄ (**a**), 3–4% KOH (**b**), 3–4% NaOH (**c**)

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Sulfonation of BA by $\text{NH}_2\text{SO}_3\text{H}$ in the presence of urea in DMF or 1,4-dioxane formed the ammonium salt of BA 3-sulfate (**2**), which was treated with H_2SO_4 (10%) to give BA 3-sulfonic acid (**3a**); with KOH or NaOH solution (3–5%), the stable salts **3b** or **3c**.

IR spectra of **3b** and **3c**, in contrast to that of BA, showed an absorption band at 830 cm^{-1} (SO), and a strong band at 1221 cm^{-1} (SO_2), which confirmed that **3b** and **3c** contained sulfate groups [11] in addition to **2** and **3a**.

^{13}C NMR spectra of the natural 3α - and 3β -stereomers of BA and 3α - and 3β -stereomers of BA sulfates isolated from *Schefflera octophylla* leaves were reported [11] and showed that the C-3 resonance in 3α -BA was observed at 75.5 ppm; in 3β -BA, at 78.2 ppm. The C-3 resonance of BA 3α -sulfate had a chemical shift of 82.9 ppm; of BA 3β -sulfate, 86.0 ppm.

A comparison of the ^{13}C NMR spectra of starting BA and sulfates **3b** and **3c** showed that replacing the OH by SO_3H shifted the C-3 resonance from 79.0 to 86.5 ppm.

Thus, it was established that reaction of BA and $\text{NH}_2\text{SO}_3\text{H}$ in the presence of urea in DMF and 1,4-dioxane occurred in 2.5–3.5 h at 65–75°C. The yield of **2** was 96%; **3a**, 94; **3b**, 93; **3c**, 95.

The advantage of BA sulfonation by $\text{NH}_2\text{SO}_3\text{H}$ is the use of more available and less aggressive reagents. The nature of the solvent (DMF and 1,4-dioxane) did not substantially affect the course of BA sulfonation.

EXPERIMENTAL

General. IR spectra were taken from KBr pellets (3 mg of sample/300 mg of KBr) on a Tensor 27 FTIR spectrometer (Bruker, Germany) in the range $400\text{--}4000\text{ cm}^{-1}$. ^{13}C NMR spectra were recorded in CD_3OD with TMS = 0 on a Bruker Avance III spectrometer (600 MHz). Elemental analysis with simultaneous determination (%) of C, H, N, S, and O was performed on a FlashEATM 1112 analyzer (Thermo Quest, Italy). The K and Na contents were determined on an AAnalyst-400 atomic absorption spectrometer (PerkinElmer) using an acetylene/nitrous-oxide flame. Melting points were measured on an Electrothermal A9100 apparatus.

Betulonic acid (**1**) was prepared from betulin by the known method in two steps. The first consisted of betulin oxidation by CrO_3 in AcOH to betulonic acid (61% yield, mp 247–249°C) [1]. The second involved betulonic acid reduction by NaBH_4 in THF to betulonic acid (94% yield, mp 290–292°C) [1, 12, 13]. The BA used for sulfonation was the single β -stereomer. This was confirmed by the full agreement of its physicochemical properties with those published [7, 11–13]. DMF was dried by shaking with KOH and distilled over CaO. 1,4-Dioxane was dried over KOH and distilled over Na [14]. Salts **3b** and **3c** were purified by recrystallization from MeOH (75%).

Sulfonation of BA in DMF and 1,4-Dioxane. Ammonium 3β -O-Sulfate-lup-20(29)-en-28-oate (2**).** DMF or 1,4-dioxane (50 mL) in a 100-mL three-necked flask equipped with a stirrer and thermometer was stirred vigorously; treated with $\text{NH}_2\text{SO}_3\text{H}$ (1.46 g, 0.015 mol), urea (0.90 g), and **1** (4.56 g, 0.01 mol); heated on a water bath at 65–75°C for 2.5–3.5 h, cooled, diluted with H_2O (100 mL), transferred to a separatory funnel, and extracted with BuOH (130–150 mL). The BuOH extract was washed with H_2O and evaporated to dryness *in vacuo*. Yield of **2**, 96%, mp (dec.) 147–150°C. $\text{C}_{30}\text{H}_{54}\text{N}_2\text{O}_6\text{S}$.

3β -O-Sulfate-lup-20(29)-en-28-oic Acid (3a**).** The BuOH extract containing **2** was acidified with H_2SO_4 solution (10%) to pH 2–3. The BuOH layer was separated and concentrated *in vacuo* to afford **3a** in 94% yield, mp (dec.) 139–143°C, lit. mp 140–142°C [14]. $\text{C}_{30}\text{H}_{48}\text{O}_6\text{S}$.

Potassium 3β -O-Sulfate-lup-20(29)-en-28-oate (3b**).** The BuOH extract containing **2** was worked up with KOH solution (3–4%) to pH 8–9. The BuOH layer was separated and concentrated *in vacuo* to afford **3b** in 93% yield; 74% after recrystallization, mp (dec.) 312–315°C. $\text{C}_{30}\text{H}_{46}\text{O}_6\text{SK}_2$. IR spectrum (KBr, ν , cm^{-1}): 838 (C–O–S), 1221 (O=S=O). ^{13}C NMR spectrum (CD_3OD , δ , ppm): 38.5 (C-1), 24.1 (C-2), 86.4 (C-3), 38.6 (C-4), 56.1 (C-5), 18.1 (C-6), 34.7 (C-7), 40.1 (C-8), 51.1 (C-9), 37.2 (C-10), 20.9 (C-11), 26.5 (C-12), 38.5 (C-13), 42.4 (C-14), 31.4 (C-15), 32.0 (C-16), 56.5 (C-17), 47.1 (C-18), 48.2 (C-19), 151.1 (C-20), 31.3 (C-21), 37.6 (C-22), 28.4 (C-23), 16.6 (C-24), 16.2 (C-25), 16.1 (C-26), 14.7 (C-27), 179.0 (C-28), 20.1 (C-29), 109.3 (C-30).

Sodium 3β -O-Sulfate-lup-20(29)-en-28-oate (3c**).** was prepared analogously to **3b** using NaOH solution (3–4%) in 95% yield, 76% after recrystallization, mp (dec.) 314–317°C. $\text{C}_{30}\text{H}_{46}\text{O}_6\text{SNa}_2$. The IR and ^{13}C NMR spectra of **3c** were identical to those published [15].

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