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Allylic nitration of 3β-sitosterol and cholesterol acetate: Preparation of 7-nitro derivatives

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The reaction of 3-β-sitosterol and cholesterol acetates with a HNO_3/bentonite system using infrared or microwave radiation as the energy source leads to 7-nitro derivatives as major products. (*Steroids* **62**:500–503, 1997) © *1997 by Elsevier Science Inc.*

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Introduction

Nitrosterols and other nitrosteroidal compounds have been prepared from such nitrating reagents as HNO_3, N_2O_4, NOCl.[1–4] The addition reaction of nitrating species upon the double bond in C_1-C_6 gives 6-nitro-en-derivatives as the principal products. Only a few nitration reactions yielding the 7-positioned product have been reported.[5]

Recently, reagents supported over inorganic materials[6] have been advantageously employed in organic synthesis. For example, the use of a Mexican bentonitic clay has been reported in the oxidation of 1,4-dihydropyridines with the HNO_3/bentonite[7] or the MnO_2/bentonite systems[8,9] and with microwave radiation as the energy source.

Here, we disclose our main results obtained from the reaction of 3β-sitosterol and cholesterol acetates with the HNO_3/bentonite system, using infrared or microwave radiation as the heat source (Figure 1).

Experimental

Melting-point (mp) determinations were performed in a Fisher–Johns apparatus and are uncorrected. Infrared spectra were determined on a Perkin–Elmer 283 IR-spectrometer or a Nicolet FT-IR 55X using chloroform as solvent. ^{1}H NMR spectra were recorded at 200 MHz using Varian Gemini-200 and Varian VXR-300S spectrometers, with TMS as the internal reference and deuterochloroform as the solvent. Mass spectra were recorded on a Hewlett-Packard 5985-B mass-spectrometer (70 eV).

Characterization of the bentonite clay

On examination by X-ray fluorescence, Salmón et al.[10] found the clay employed in this study to have the following composition (in %): SiO_2, 75.4, Al_2O_3, 9.3; MgO, 0.4; Fe_2O_3, 1.3; CaO, 4.0; K_2O, 0.4; TiO_2, 0.4; H_2O (110°) 9.5. The commercial acid-activated material was obtained from Tonsil Mexicana and analyzed with a Phillips spectrometer using Cr primary radiation. The measured specific surface area was 307 m^2/g (B.E.T. N_2), and the pore volume was 0.4789 cm^3/g. The acidity by NH_3 thermodesorption was 0.099 meg/g. The particle size was 325 mesh.

Preparation of the supported reagent (HNO_3/bentonite system)[7]

A mixture of concentrated HNO_3 (100 mL) and bentonitic clay (50 g) was stirred for 24 h and was subsequently filtered under vacuum until a dry powder was obtained.

*Preparation of β-sitosterol and cholesterol acetates (**1a** and **1b**)*

1a and **1b** were prepared in quantitative yields according to standard and well-established procedures.[11]

Nitration of β-sitosterol acetate (microwave radiation)

Compound **1a** (0.3g) and the HNO_3/bentonite system (1.5g) were homogenized, and the resulting mixture was put in a reaction tube

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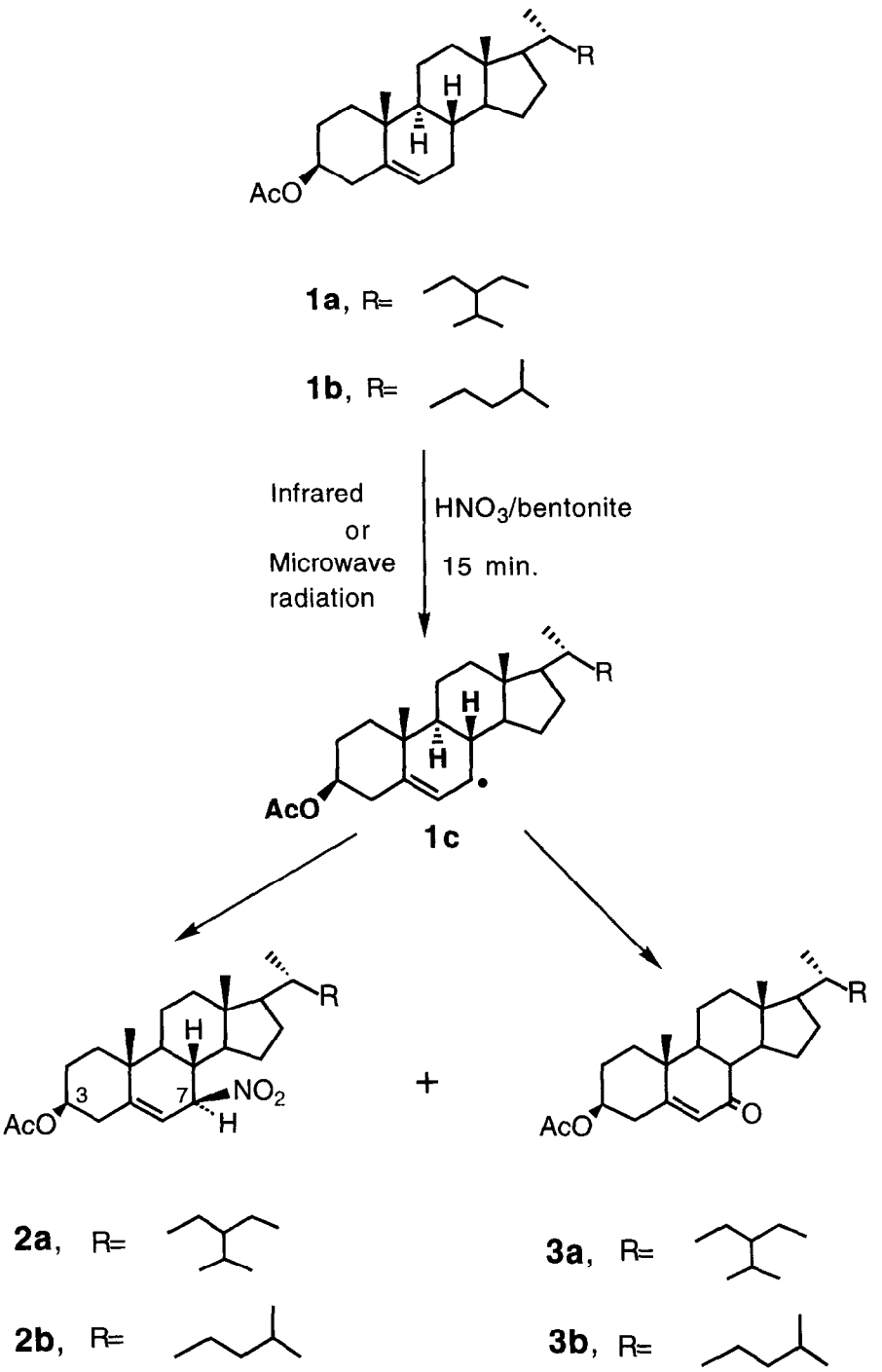

Figure 1. Structure of compounds.

with a trap to collect the nitrogen oxides generated during the reaction. The tube was irradiated three times (9 min each time) in a conventional microwave oven (2450 MHz) at the maximum potential, about 175°C. The mixture was then extracted with AcOEt, filtered, and the solvent was removed under reduced pressure to yield a yellow oil. The oil was purified by silica gel chromatography with petroleum ether/AcOEt (95:5) as the eluent to give 0.063 g (20%) of a pure yellow oil (**2a**), which was visualized as a blue spot when chromatographed from a 1% ceric sulfate solution with petroleum ether/AcOEt (9:1) as the eluent (R_f = 0.39). The other fractions contained recovered **1a**.

Nitration of β-sitosterol acetate (infrared radiation)

Compound **1a** (3g) and 22.5 g of the HNO_3/bentonite system were homogenized, and the mixture was put in a round-bottomed flask fitted with a reflux condenser and stirred magnetically. The flask was irradiated with a commercial infrared lamp (250 W) for 45 min.[12] The reaction mixture was extracted with AcOEt and washed with 10% $KHCO_3$ (25 mL) and water (30 mL). After drying with Na_2SO_4, the solvent was removed under reduced pressure to yield a wine-colored oil residue, which was purified as above to give 0.46 g (14%) of (**2a**), a yellow oil. I.R. $\nu_{max.}$ (cm^{-1}): 1955, 1470

(-CH_3); 1465, 2871 (-CH_2-); 1726 (ester C=O); 1671 (trisubstituted C = C); 1550, 1370 (-NO_2); 1250 (C-O); 1034 (CO-O). ^{1}H RMN δ 5.3 (1H, d, 3.7 Hz, H-6); 4.9 (1H, d, 9.1 Hz, H-7); 4.6 (1H, m, H-3); 2.5 (1H, m, H-8); 2.4 (2H, d, H-4); 2.02 (3H, s, CH_3-acetate), 1.16, 0.71 (6H, 2s, Me-19 and Me-18), 0.72–0.9 (12 H, d and s, 4 Me of the chain). MS-EI, M/z (relative intensity in %): M^+ 501, 412 (1.3), 395 (33), 57 (34), 55 (37), 43 (100). HR-MS (+FAB), M^+501. Anal. calc. for $C_{31}H_{51}N$: C,71.91; H,9.93; N, 2.71. Found: C; 72.50; H, 10.10; N, 2.72.

The more polar fractions gave 0.18 g (6%) of a white solid (**3a**), which was recrystallized in methanol (mp 159–160°C) and appeared as a yellow-orange spot when chromatographed from a 1% ceric sulfate solution with a R_f=0.56 using petroleum ether/AcOEt (8:2) as eluent; I.R. $\nu_{max.}$ (cm^{-1}): 1728 (ester C=O), 1667 (α,β-unsaturated ketone). ^{1}H RMN δ 5.71 (1H, bs, C=CH-, C-6), 4.72 (1H, m, H-3) 2.53 (1H, bd, H-4), 2.05 (3H, s, $C\underline{H}_3$ acetate), 1.2 (3H, s, Me-19), 0.7 (3H, s, Me-18), 0.75–1.0 (12H, 4 Me of the chain). EIMS (*m/z*) (relative intensity in %): 410 (80) (M^+-60), 395 (50), 174 (60), 43 (100).

Nitration of cholesterol acetate

In a similar way (*vide supra*), the cholesterol acetate was allowed to react with the HNO_3/bentonite system under microwave and infrared energy and yielded **2b** (18%); I.R. $\nu_{max.}$ (cm^{-1}) 1726 (ester C=O); 1671 (trisubstituted C=C); 1550, 1370 (-NO_2). ^{1}H RMN ($CDCl_3$) δ 5.3 (1H, d, J=3.7 Hz, H-6); 4.9 (1H, d, J=9.1Hz, H-7); 4.6 (1H, m, H-3); 2.5 (1H, m, H-8); 2.4 (2H, d, H-4); 2.01(3H,s, $C\underline{H}_3$ acetate), 1.16, 0.71 (6H, 2s, Me-19 and Me-18), 0.90 (3H, d, J=7Hz, Me of the chain), 0.85 (6H, d, J=7Hz, 2Me of the chain).

A second product (**3b**) was obtained as a white solid, mp 157–60°C, in a poor yield (less than 1%). I.R. ν_{max} (cm^{-1}): 1728 (ester C=O), 1667 (α,β-unsaturated ketone). ^{1}H RMN δ 5.71 (1H, bs, C=CH-,C-6), 4.72 (1H, m, H-3) 2.53 (1H, bd, H-4), 2.05 (3H, s, $C\underline{H}_3$ acetate), 1.2 (3H, s, Me-19), 0.7 (3H, s, Me-18), 0.75–1.0 (9H, 2d, 3 Me of the chain). EIMS M/z (relative intensity in %): 382 (80) (M^+-60), 367 (50), 43 (100).

Results and discussion

The reaction of β-sitosterol acetate (**1a**) and the HNO_3/bentonite system using microwave or infrared radiation led to a 3:1 mixture (25%) of two compounds whose structures were established by spectroscopic techniques. The most abundant product was identified as 7α-nitro-3β-sitosterol acetate (**2a**) according to the molecular formula established by HRMS (+FAB) (high-resolution mass spectrometry plus fast atom bombardment). The infrared spectrum revealed a band at 1755 cm^{-1} corresponding to a carbonyl group of an ester function a band of a trisubstituted double bond at 1671 cm^{-1}, and two bands at 1550 and 1370 cm^{-1}, which correspond to the nitro group. The ^{1}H NMR spectrum demonstrated a signal from the vinylic proton of C-6 at 5.3 ppm (1H, bd; J=1.61 Hz), a double signal at 4.6 ppm (H, m) attributable to the base proton of the ester function in C-3 and a multiple signal at 2.4 ppm (2H, m) because of the methylene in C-4. A tripleted double signal centered at 4.9 ppm (1H, td, J=9.16 and J=1.6 Hz) was assigned to the proton on C-7 bearing the nitro group. The signal of the C-8 proton is shifted to 2.5 ppm (1H, d, J=9.1 Hz). A single signal is also noticed at 2.05 ppm corresponding to the methyl group of the acetate function, along with the single signals of five methyl groups. Coupling constants of the proton on C-7 allow for the determination of the stereochemistry of this proton with respect to that on C-8; these protons are in an *axial–equatorial* orientation, and therefore, H-7 has α orientation making the C_7-nitro substituent β-oriented.

The assignment for each of the carbons of the reaction products is presented in Table 1. We can observe that the shift of C-4 in **2a** and **2b** does not considerably differ from that observed in **1a** and **1b**, respectively. This fact suggests that the methylene in C-4 has a similar surrounding magnetic environment in each case. The C-7 shift (91.17 ppm) in **2a** presents a great change (ca. 60 ppm) with respect to that in **1a** due to the presence of the nitro group. Furthermore, the shifts of C-5 and C-6 are also influenced by the nitro group function, as are the shifts of C-8 and C-9. All of the results were corroborated by distortionless enhancement by polarization transfer (DEPT) experiments, indicating the presence of six primary carbons, one secondary carbon less than in **1a**, and one tertiary carbon more than the same substrate. APT experiments provided further evidence for the proposed structure **2a**. By the same token, correlated spectroscopy (COSY) experiments allowed for the observation of interactions of H-7 with H-6 and H-8. The less abundant product was assigned the structure of ketone **3a**, which had spectroscopic data and physical constants identical in all respects to those reported in the literature.[13]

The reaction of cholesterol acetate with HNO_3 supported on bentonite resulted in two products that were characterized by spectroscopic techniques. The major component of the reaction mixture was identified as 7α-nitro-3β-cholesterol acetate (**2b**). The infrared spectrum displayed the bands corresponding to the carbonyl group, the double bond in C-5 and C-6, and the nitro group at 1550 and 1370 cm^{-1}. The ^{1}H NMR spectrum disclosed the signals for the protons on C-3, C-4, C-6, and C-8, which did not differ with respect to those of **2a**, but, in this case, we observed a double signal at 4.9 ppm belonging to the proton on C-7. We repeated NMR experiments for this compound, as in the case of **2a**, and the proposed structure was confirmed. The second compound was assigned the structure of the ketone **3b** based on spectroscopic analysis and physical constants reported previously.[13]

It must be mentioned that control experiments involving the heating of steroid-HNO_3/bentonite mixtures in a water and/or oil bath (i.e., without microwave or infrared radiation as the heat source) were carried out, and there was no reaction at all in any case, even when the final temperature matched those in our current experiments (i.e., 175°C).

The allylic nitration of C-7 in **1a** and **1b** can be explained by the possible formation of $\cdot NO_2$ free radicals generated by the microwave or infrared radiation in the HNO_3/bentonite system, which catalyzes the reaction. This assumption is supported by the position involved in the nitration reaction (i.e., allylic position) and by evidence in the literature[14] reporting $\cdot NO_2$ free radicals involved in selective nitrations by "claycop," an acidic montmorillonite clay. It is also formed an allylic free radical **1c** at the same time, which explains the formation of **3a** and **3b**. Formation of carbonylic compounds has been reported in α-hydrogen elimination reaction of nitrate derivatives.[15]

In conclusion, our report describes a new method to

Table 1 Chemical shifts in ^{13}C NMR for **1a, 2a, 2b,** and **3a** products

No. C	1a	2a	2b	3a
1	36.97	36.05	35.58	35.99
2	27.75	27.44	27.42	27.34
3	73.97	72.65	72.66	72.20
4	38.10	37.47	37.46	37.73
5	139.67	148.48	148.54	163.85
6	122.67	117.97	118.01	126.70
7	31.85	91.17	91.19	201.98
8	31.85	35.60	35.95	49.79
9	50.02	47.51	47.50	49.93
10	36.56	36.21	35.99	36.22
11	21.00	21.26	21.24	21.16
12	28.22	27.98	27.98	28.53
13	42.30	42.98	42.97	43.10
14	56.68	55.73	55.72	54.67
15	24.26	23.78	23.05	26.30
16	39.70	39.14	39.12	38.64
17	56.04	55.39	55.27	54.67
18	11.95	11.81	11.78	11.96
19	19.28	18.88	18.85	28.53
20	36.13	35.60	35.95	35.99
21	18.76	18.68	18.85	18.91
22	33.91	36.22	33.82	33.91
23	— —	— —	— —	— —
24	45.81	39.43	45.78	45.81
25	— —	— —	— —	— —
26	— —	— —	— —	— —
27	29.12	22.76	29.10	29.11
28	19.01	— —	18.72	19.01
29	— —	— —	— —	— —
30	170.57	170.36	170.45	170.28
31	21.42	20.99	20.96	21.16

nitrate steroid derivatives, providing an alternative option for mild and selective synthesis of this kind of compound. Although the yields are not good so far, work is in progress to improve our results and increase knowledge of the mechanisms involved.

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