# NOTE

## PREPARATION OF |2,4-$^{2}$H|- AND |2,4-$^{3}$H|-TOMATIDINE

D. Doller and E. G. Gros

Departamento de Química Orgánica y UMYMFOR, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

SUMMARY

The preparation of |2,4-$^{2}$H|- and |2,4-$^{3}$H|-tomatidine was accomplished by oxydation of the unlabelled compound to tomatidone, exchange of the labile hydrogens by reaction with $^{2}H_2O$ and $^{3}H_2O$ on basic alumina and direct reduction of the labelled tomatidone to tomatidine. The efficiency of the procedure was checked by physical ($^{1}$H and $^{13}$C NMR, MS) methods.

Key Words: |2,4-$^{2}$H|-Tomatidine, |2,4-$^{3}$H|-Tomatidine

In connection with our project on biodegradation of alkaloids in plants (1-5) we needed radioactive tomatidine labelled at the ring A. For this purpose we attempted the exchange reaction of hydrogen atoms at enolizable positions vicinal to carbonyl groups. Thus, by modification of a reported method (6), tomatidine (1) was oxydized to tomatidone (2) which, in turn, was treated with $^{2}H_2O$ or $^{3}H_2O$ on basic alumina (7) to afford deuterated- and tritiated-tomatidone respectively. In the former case the efficiency of the labelling process was ascertained by mass spectroscopic analysis whilst radioactivity measurement was used in the latter. The reconvertion of tomatidone into tomatidine was accomplished by two procedures. On one hand, treatment of 2 with lithiumaluminum hydride in ethyl ether afforded dihydrotomatidine A (3). As it has been claimed (8) that dihydrotomatidine A could be converted into tomatidine by oxydation with activated manganese dioxide, we attempted this reaction but failed to reproduced the reported results; therefore, the convertion of 3 into 1 was performed through the N-chloro-dihydrotomatidine A (4) as it has been described elsewhere (9). On the other hand and in order to avoid the opening of the ring E when going through compound 3, tomatidone was directly reduced with aluminum isopropoxide in isopropanol yielding in one step a mixture of tomatidine and

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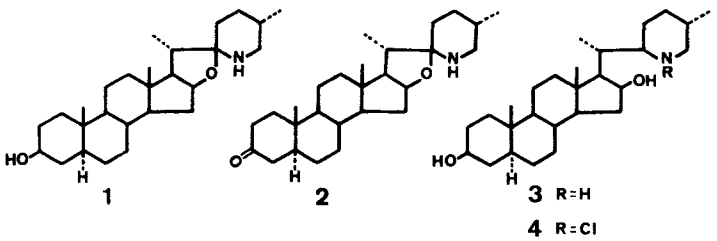
its 3α-epimer with a relation 3β/3α of 3:1 as determined by the $^{13}C$ NMR spectrum of the mixture. This could be separated by preparative TLC. By application of this procedure the preparation of tomatidine from labelled tomatidone was achieved in 52% yield with 65-70% of retention of the label.




## EXPERIMENTAL

Melting points were determined with a Fischer-Johns hot plate and are uncorrected. $^{1}H$ and $^{13}C$ FTNMR were recorded at 100 and 25.2 MHz respectively with a Varian XL-100-15 spectrometer. Mass spectra were registered at 70 eV (direct inlet) with a Varian-Mat CH7-A spectrometer interfaced to a Varian-Mat Data System 166 computer. Radioactivity was measured by liquid scintillation counting. $^{2}H_2O$ was purchased from Merck, Sharp & Dohme, Canada; $^{3}H_2O$ was obtained from the Comisión de Energía Atómica, Argentina.

*Tomatidone* (2). Tomatidine (1, 100 mg) was treated with anhydrous HMPT (0.48 ml), $CH_2Cl_2$ (0.48 ml) and DMSO (0.18 ml) and the mixture was cooled to - 20°C. A solution of methansulphonic anhydride (83.4 mg) in $CH_2Cl_2$ (0.3 ml) was added and the reaction mixture was left aside at - 15°C for 5 days. The reaction was quenched by addition of $Et_3N$ (0.19 ml), warmed to room temp. with occasional shaking and poured into ice-water where the oily product crystallized on stirring. The product (90.6 mg) was purified by medium-pressure liquid chromatography on silica gel H eluting with $CH_2Cl_2$-MeOH-$NH_4OH$ (99:1:0.2) yielding unreacted tomatidine (22.0 mg) and pure tomatidone (61.9 mg; 62%) of m.p. 201-203°C (MeOH); lit (10) m.p. 195-197°C. $^{1}H$ NMR ($CDCl_3$-TMS): δ 0.86 (3H, s, Me-18), 0.87 (3H, d, J=7 Hz, Me-21), 0.97 (3H, d, J=7 Hz, Me-27), 1.04 (3H, s, Me-19), 2.75 (2H, d, J=7 Hz, H-26), 4.16 (1H, m, H-16). $^{13}C$ NMR ($CDCl_3$-TMS): δ 11.5 (C-19), 15.8 (C-21), 16.9 (C-18), 19.3 (C-27), 21.2 (C-16), 26.6 (C-23), 28.5 (C-24), 28.8 (C-6), 31.0 (C-25), 31.9 (C-7), 32.6 (C-15), 34.9 (C-8), 35.7 (C-10), 38.0 (C-2), 38.5 (C-1), 40.0 (C-12), 40.8 (C-13), 43.0 (C-20), 44.6 (C-4), 46.6 (C-5), 50.2 (C-26), 53.8 (C-9), 55.5 (C-14), 61.9 (C-17), 78.3 (C-16), 99.0 (C-22), 211.4 (C-3). MS (m/z, %): 413 ($M^+$, 7), 398 (2), 385 (8), 271 (5), 138 (83), 114 (100).

*Deuterated tomatidone.* A solution of compound 2 (10 mg) in benzene-hexane (3:2, 0.2 ml) was filtered through a column of basic alumina (3.3 g) previously equilibrated with $^2H_2O$ (99.5%, 0.1 ml) which was eluted with the same solvent mixture at a rate of 30 drops/min. The deuterated tomatidone thus obtained (10 mg) presented an isotopic distribution of M+3: 0.8%, M+2: 10.6%, M+1: 40.8%, M: 47.8% as determined by mass spectroscopy analysis and calculated by a computation program (11). The mass spectrum also showed the presence of deuterated positions at the F ring.

*Tritiated tomatidone.* In a typical experiment compound 2 (3.5 mg) was treated as described above with $^3H_2O$ (35 mCi) on basic alumina (3 g) affording radioactive tomatidone (3.5 mg) of sp. act. 1.0 mCi/mmol.

*|2,4-$^2$H|-Tomatidine.* Deuterated tomatidone (15.1 mg) was treated with aluminum isopropoxide (100 mg) in *i*-PrOH (1 ml) and refluxed for 1 hr. Water (1 ml) was added, the mixture was made alkaline by addition of 5N NaOH solution (1 ml) to exchange deuterium atoms other than at positions 2 and 4, and it was extracted with ethyl ether (3 x 15 ml). The organic layer was washed with water and dried over $MgSO_4$. Removal of the solvent afforded 15.0 mg (99%) of the isomeric mixture at C-3 which was resolved by preparative TLC on silica gel 60 $F_{254}$ developing thrice with $CH_2Cl_2$-$Et_3N$ (99:1). This procedure yielded pure tomatidine (9.0 mg, 59%) of m.p. 204-208°C (MeOH), lit. (12) m.p. 203-208°C, and 3.0 mg of the α-isomer. Mass spectroscopic analysis indicated a distribution of M+2: 1.8%, M+1: 39.8%, M: 58.4% and absence of isotopic labelling at ring F. The net retention of the label at ring A resulted of 67.4%.

*|2,4-$^3$H|-Tomatidine.* Tritiated tomatidone (8.7 mg, 1.0 mCi/mmol) was treated with aluminum isopropoxide (100 mg) in *i*-PrOH (1 ml) as indicated above. Following the previously described procedure radioactive tomatidine (4.5 mg, 0.64 mCi/mmol) of m.p. 205-208°C (MeOH) was obtained.

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