HIGH SPECIFIC ACTIVITY TRITIUM LABELLED O-BENZOYL ECGONINE

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SUMMARY

Through the use of an O-(o-iodobenzoyl) ecgonine derivative (3), tritium labelled O-benzoyl ecgonine has been prepared, by catalytic hydrogenolysis, in sufficiently high specific activity for use in radioimmunoassay studies for cocaine metabolites. The ease of converting O-benzoyl ecgonine to cocaine also makes this labelled substrate potentially useful in metabolism studies of the latter.

Key words: O-Benzoyl ecgonine-^{3}H, O-Benzoyl ecgonine-^{2}H, O-(o-iodobenzoyl ecgonine-^{2}H, O-(o-iodobenzoyl) ecgonine.

For the development of a radioimmunoassay[1] for biotransformed cocaine (1), a suitably labelled metabolite was required. While little is known regarding the biotransformation of 1, it is believed[1,2] that the hydrolysis product, O-benzoyl ecgonine (2), is the major urinary metabolite. In this connection, we report the preparation of specifically tritiated 2 of sufficiently high activity for use in radioimmunoassay studies. In addition, since 2 is easily converted to 1, the position of the label as well as the level of radioactivity in this derivative should make it useful[2] in studies of the metabolism of 1.

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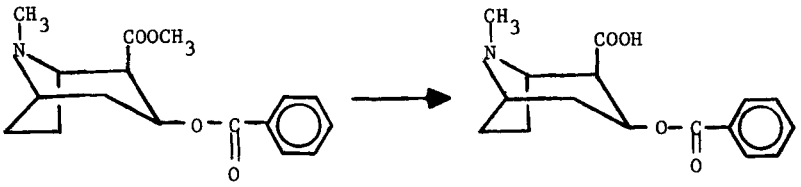
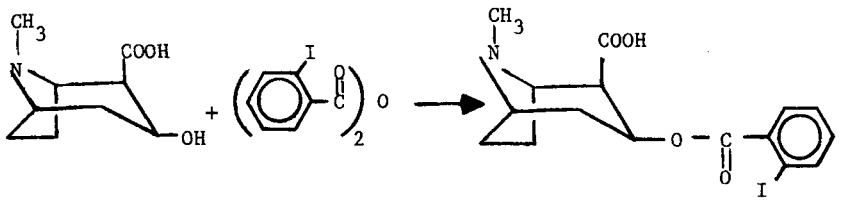


1, cocaine 2, O-benzoyl ecgonine

While several literature references[3,4,5] describe the tracer level tritium labelling (and ^{14}C labelling[6,7]) of cocaine itself, there is no precedent for the high level labelling of this substrate or even a tracer level labelling of its metabolite (2). Two references describing the random exchange of 1 (Wilzbach[3] and acid catalyzed-exchange[4]) report specific activities of 3.6 μCi/mg and 630 μCi/mg, respectively. Benzoyl labelled 1 has been prepared[5] by the condensation of tritium labelled benzoyl chloride with methyl ecgonine, though this was accomplished in even lower specific activity (0.539 μCi/mg). Methyl ester labelled cocaine has also been prepared[8] with a specific activity of 311 μCi/mg. A method for the stable labelling (deuterium) of 1 and 2 has been reported[9] but is not adaptable to radio-labelling.

Several attempts to improve upon these general exchange techniques[3,4] (using deuterium) failed to generate 2 with any reasonably high isotope incorporation. It was apparent that any high specific activity synthesis would require a suitable precursor into which gaseous tritium could be introduced by catalytic reduction. For this reason, we decided to prepare the aryl-iodo derivative 3. Condensation[10] of ecgonine (4) with

o-iodobenzoic anhydride (5) (prepared by standard procedures) gave 3. Hydrogenolysis of 3 with deuterium (using 10% palladium on carbon in methanol) gave 2a (mass spectrum: d_o = 6.3%, d_1 = 93.7%). Reduction of 3 with tritium gas gave 2b having a specific activity of 6.01 Ci/mmol (20.8 mCi/mg).



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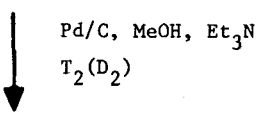

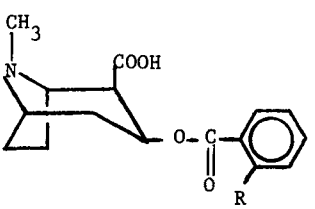

2a, R=D

2b, R=T

EXPERIMENTAL

General. - Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Spectra were recorded on standard instruments by the staff of the Physical Chemistry Department and microanalyses were performed by the Microchemical Laboratory, both of Hoffmann-La Roche Inc. Radiochemical purity was determined on thin-layer chromatograms with a Packard Model 7201 Radiochromatogram Scanner System and radioactivity was measured by the liquid scintillation technique with a Packard Tricarb Model 2010 spectrometer.

o-Iodobenzoic anhydride (5). Thionyl chloride (3.6 g, 2.2 ml, 30 mmol) was slowly added to a solution of o-iodobenzoic acid (4.96 g, 20 mmol) in 50 ml of dry benzene. The solution was briefly brought to reflux temperature (allowing a few ml to boil off) and then allowed to cool. A solution of o-iodobenzoic acid (4.96 g, 20 mmol) in pyridine (6 ml) was then slowly added. After briefly heating the solution to reflux again, the entire reaction mixture was poured onto ice and washed (twice) with dilute hydrochloric acid, twice with saturated sodium bicarbonate solution, and finally with brine. After being dried ($MgSO_4$) and concentrated in vacuo, the crude residue (9.5 g) crystallized on standing. Recrystallization from ether yielded 6.5 g of 5, mp 74.5-75.5°; ir 1798, 1737 cm^{-1}, coupled anhydride; nmr δ 7.23 and 7.47 (4H, triplets, J = 5Hz, meta aromatics) ,7.98 and 8.08 (4H, doublets, J = 5Hz, ortho aromatics); mass spectrum 315 (M^+ - I).

Anal. Calc. for $C_{14}H_8I_2O_3$: C, 35.18; H, 1.68; I, 53.10. Found: C, 35.40; H, 1.69; I, 53.07.

O-(o-iodobenzoyl) ecgonine (3). A solution of ecgonine (185 mg, 1 mmol) and 5 (956 mg, 2 mmol) in aqueous acetone (1.5 ml H_2O + 4.5 ml acetone) was stirred for 4 days at room temperature. Evaporation of the solvent *in vacuo* and purification of the residue by preparative thin layer chromatography (silica gel, 2 mm; ethyl acetate, acetic acid, water, 4:4:1) gave 166 mg (40%) of 3, mp 198-202° (decomp); ir 1727 cm^{-1} (ester carbonyl), 1600 cm^{-1} (carboxyl carbonyl), 750 cm^{-1} (ortho disubstituted aromatic); nmr δ 2.1-2.7 (6H, complex, methylenes), 2.73 (3H, s, N-CH_3), 3.96 (2H, broad, bridgehead hydrogens), 5.46 (1H, doublet of triplets, J = 3.5 and 6.5 Hz,-CHO-), 7.24 and 7.45 (2H, triplets, J = 5Hz, meta aromatics), 7.99 and 8.08 (2H, doublets, J = 5Hz, ortho aromatics); mass spectrum 415 (M^+).

O-Benzoyl ecgonine-^3H (2b). O-(o-iodobenzoyl)ecgonine (3, 15 mg, 0.036 mmol) and triethylamine (4.9 µl, 3.64 mg, 0.036 mmol) were dissolved in 0.5 ml of dry methanol in a system having a capacity of 3.5 ml. 10% Palladium on carbon catalyst (5 mg) was added. After evacuation, approximately 2 Curies of carrier free tritium gas were admitted (0.036 mmol, about 0.73 ml) and the system was first isolated, then stirred at room temperature 1 hr. Any unreacted tritium gas was then removed and 1 ml of methanol was added. The catalyst was filtered off and the filtrate concentrated to dryness *in vacuo*. Four such concentrations (to remove labile activity), from 1 ml of methanol each, gave a white residue which was purified by preparative thin layer chromatography (silica gel, 2 mm, 5% ammonium hydroxide in methanol). 4.1 mg (85.27 mCi) of 2b were isolated having a specific activity of 20.80 mCi/mg

(6.01 Ci/mmol) and a radiochemical purity of > 99% (tlc: silica gel; methanol).

A similar experiment, carried out with deuterium gas, gave 2a which contained 93.7% d_1 and 6.3% d_o (mass spectrum and nmr).

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