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Labelled Compounds of Potential Biological Interest

I. Pyrazole-4-^{3}H and 4-Methylpyrazole-3-^{3}H

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In previous studies [1,2] it was found that series of substituted pyrazoles were extremely potent inhibitors of liver alcohol dehydrogenase (LADH). 4-Bromo- and 4-iodopyrazole were the most active compounds and slightly more potent than 4-methylpyrazole. It was found [3] in our laboratories that 4-methylpyrazole was better tolerated than the two halogenated derivatives. Extended toxicity studies with 4-methylpyrazole revealed no changes in hematology, blood chemistry, urine analysis, and histopathology related to the drug treatment. The compound had thus met the criteria for commencing investigations in man.[4]

In order to carry out distribution and metabolism studies it was necessary to prepare labelled 4-methylpyrazole. For comparison we have also labelled pyrazole — another inhibitor of the ethanol oxidation.[1,2,5,6] Catalytic dehalogenation in presence of tritium appeared to be the simplest route to the labelled compounds. In trial runs the conditions were established which would cause hydrogenolysis but no hydrogenation to the pyrazolines.

Iodination of pyrazole and 4-methylpyrazole gave the desired intermediates which, on deiodination by tritium in a tritium-hydrogen gas in the presence of palladium on charcoal catalyst, furnished the labelled compounds.

The products were isolated as their oxalates and the radiochemical purity was verified by thin layer chromatography.

Experimental. Pyrazole-4-^{3}H oxalate. 4-Iodopyrazole (205 mg, 1.05 mmole) prepared according to Hüttel *et al.*[7] was dissolved in 2 ml of a solution of sodium hydroxide in 90 % ethanol (80 mg NaOH, 2 mmole) and 10 % Pd on charcoal catalyst (20 mg) was added. The mixture was hydrogenated by vigorous stirring in a H_2/T_2 mixture (20 Ci T_2) in a vacuum tritiation apparatus. Hydrogenation was complete after 20 min. The catalyst was removed by filtration, washed with ethanol and the filtrate was evaporated to dryness *in vacuo*. The residue was extracted with ether. A solution of oxalic acid in ether was added to the ether extract and the pyrazole oxalate was recrystallized from methanol-ether after dilution with 100 mg of inactive pyrazole oxalate. Yield 136 mg; m.p. 191.5–192°C (d) (uncorr.); spec. act. 8.1 mCi/mg = 1.3 Ci/mmole; activity yield 5.5 %.

Radiochemical purity was checked by thin layer chromatography on silica (Merck F-254) in a chloroform-methanol (9:1) solvent system. The product showed a single radioactive peak corresponding to the reference standard pyrazole oxalate ($R_F = 0.3$).

4-Methylpyrazole-3-^3H oxalate. 4-Methyl-3-iodo-pyrazole (213 mg, 1.03 mmole) prepared according to Hüttel *et al.*[7] was dissolved in 1.5 ml of 90 % ethanol containing 60 mg of NaOH (1.5 mmole). Palladium on charcoal catalyst (10 %, 20 mg) was added and the mixture was vigorously stirred in a hydrogen-tritium mixture (37 Ci T_2) in a vacuum tritiation apparatus. The reaction was complete within 15 min. Unreacted tritium (about 10 Ci, diluted with H_2) was recovered by trapping on activated uranium powder. The catalyst was removed by filtration and washed several times with ethanol. The filtrate was evaporated to dryness *in vacuo*, the residue was extracted with ether. The ethereal solution was evaporated to a small volume (about 5 ml) and a solution of anhydrous oxalic acid (100 mg) in 10 ml of ether was added. The oxalate of the 4-methyl-3-^{3}H-pyrazole was collected and washed with ether three times. Yield 60 mg. This product was diluted with 100 mg of inactive 4-methyl-pyrazole oxalate and recrystallized from methanol-ether. Yield 150 mg; m.p. 155.5–156°C (uncorr.); spec. act. 14.7 mCi/mg=2.5 Ci/mmole; activity yield 8.7 %.

The product showed a single radioactive peak (R_F =0.21) on silica thin layer plate (Merck F-254) developed with chloroform-methanol (95:5), which corresponds to the spot of the reference standard, which was made visible by exposing the chromatogram to iodine vapours.

Radioactivity measurements. The radioactivity of the products was measured in a Packard liquid scintillation spectrometer (Model 3320) using internal standardization (Hexadecane-1,2-^{3}H). Scanning of the chromatogram was made in a Packard radiochromatogram scanner (Model 7200).

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Note on the Use of Active Esters in Combination with 1,2,4-Triazole in Solid Phase Peptide Synthesis

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In a series of papers,[1–4] Beyerman and co-workers advocated the use of bifunctional catalysts in the synthesis of peptides with the aid of active esters. This procedure was later extended by the same school [5,6] to solid phase peptide synthesis [7] (SPPS).

Since at the present state of SPPS, active esters, especially *p*-nitrophenyl esters, are generally used for incorporation of asparagine and glutamine, we recently did a quantitative study [8] of the reactivity of such an ester towards amino acids fixed to a resin. This communication reports some further experiments of the same kind wherein we have investigated the use of 1,2,4-triazole as a catalyst for the coupling reaction. Since preliminary experiments with *t*-butyloxycarbonyl-L-asparagine *p*-nitrophenyl ester (Boc-L-Asn-ONp) did not reveal any accelerating effect (Table 1), we also studied a few other active esters. In no case have we observed any improvement in coupling efficiency in the presence of triazole.

Table 1. Coupling experiments [a] with Boc-L-Asn-ONp and L-Leu-L-Ala-polymer with addition 1,2,4-triazole.

Experiment No.	Reaction time h	Yield (%) I	Yield (%) II	Yield [8] without triazole %
1	1	64	60	70
2	5	82	78	90
3	17.5	94	96	99.6

[a] Detailed reaction conditions are found under Procedure and results.

For the final experiments four active esters of Boc-L-Phe were used: *p*-nitrophenyl (—ONp), pentachlorophenyl (—PCP), *N*-hydroxysuccinimide (—OSu),