Four Syntheses Incorporating Carbon-14 or Tritium into *trans*-1-Methyl-1,4,5,6-tetrahydro-2-[2-(2-thienyl)vinyl]pyrimidine (Pyrantel, Banminth®)

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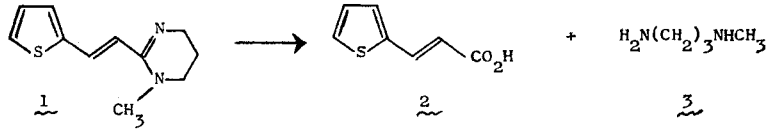
SUMMARY

Labelled pyrantel, an anthelmintic agent, was required for metabolism studies in several animal species. Four labelled syntheses are described incorporating carbon-14 or tritium into selected sites of the pyrantel molecule. The ultimate location of the marker in each synthesis was chosen in order to yield information about the metabolic breakdown patterns of pyrantel.

The title compound, pyrantel, *trans*-1-methyl-1,4,5,6-tetrahydro-2-(2-α-thienylvinyl)pyrimidine (**1**), has been shown to have a wide spectrum of anthelmintic activity in domestic animals [1] and has also been used in man [2]. In the course of metabolic studies [3] we required pyrantel labelled with radioactive tracers, and this paper describes four syntheses in which carbon-14 or tritium was incorporated into specific positions in the molecule.

During the metabolic studies use was made of the alkaline hydrolysis reaction of amidines, which when applied to pyrantel yielded 2-thiopheneacrylic acid (**2**) and N-methyl-1,3-propanediamine (**3**).

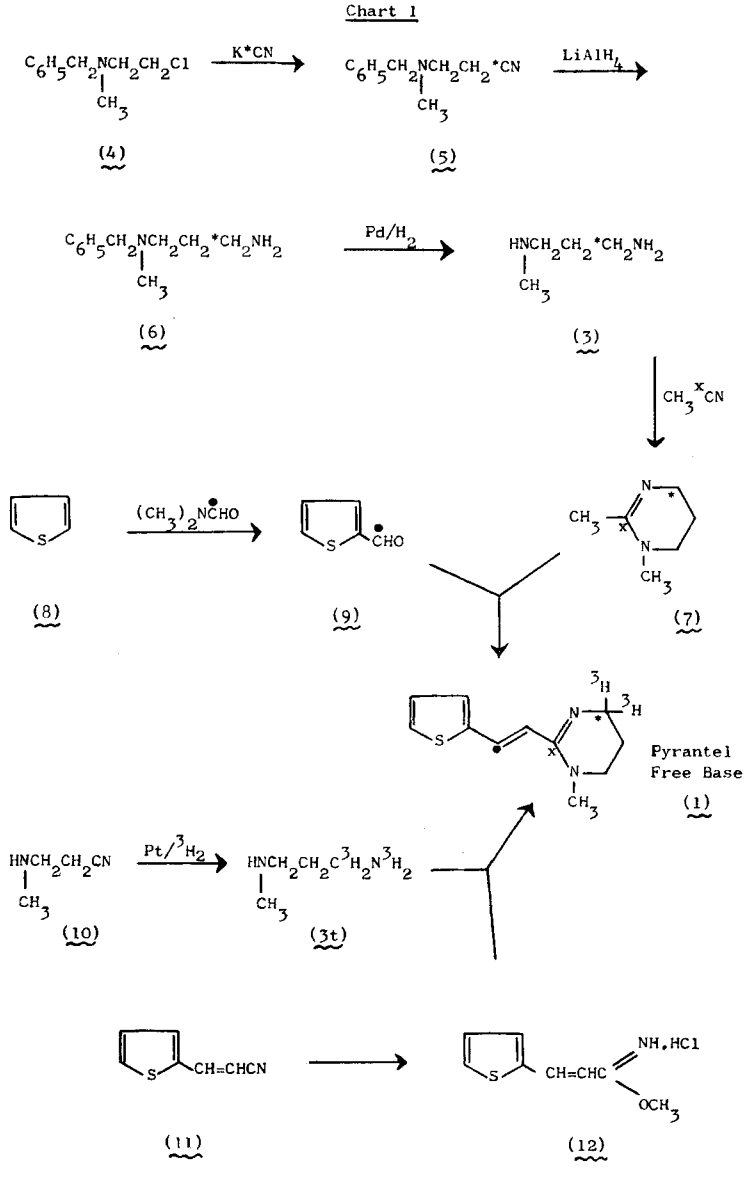


1 2 3

Metabolic modifications of pyrantel would, in most cases, give rise to compounds vulnerable to a similar hydrolysis and either **2** or **3** could be formed

as products. The quantity of each which was obtained after hydrolysis of metabolites would reflect the *in vivo* stability of the respective heterocyclic parts of the pyrantel molecule. In order to provide a method for the quantitation of the two reaction products, pyrantel was synthesised with labels located such that they would appear, after the hydrolysis of metabolites, in either **2** or **3**. Methods for isolating the products were developed and both were

Chart 1



determined by reverse isotope dilution analysis, the diamine **3** being crystallised as the hydrochloride salt.

Essentially two synthetic sequences, shown in Chart I, allowed incorporation of label into four different positions. Although isotope incorporation into four different positions is illustrated for simplicity on the one chart, only a single label incorporation was effected in each synthesis.

N-Benzyl-N-methyl-2-chloroethylamine (**4**) was treated with KCN to give the nitrile **5** in 90 % yield. Reduction of this nitrile with lithium aluminium hydride gave the primary amine **6** in 85.5 % yield and on catalytic hydrogenation over Pd at *ca* 3 atm. this amine was debenzylated to N-methyl-1,3-propanediamine (**3**) in 82.4 % yield. Reaction of this diamine with acetonitrile in the presence of phosphorous pentasulphide yielded the key tetrahydropyrimidine intermediate **7** in *ca* 75 % yield, i.e. 47.5 % overall yield from the amine **4**.

Carbon-14 was introduced either at the first stage using $K^{14}CN$, or at the cyclization stage using $CH_3{}^{14}CN$, resulting in label at the 4- and 2-positions respectively of the tetrahydropyrimidine ring.

An alternative route for the preparation of N-methyl-1,3-propanediamine is catalytic reduction of N-methyl-3-aminopropionitrile (**10**). This was carried out over platinum black in the presence of tritium gas and gave a 25 % yield of the required diamine **3t** with tritium introduced at C_3 and N_2 of the diamine moiety. This tritiated diamine (**3t**) was converted to pyrantel-^{3}H tartrate by condensation with the iminoether from 2-thiopheneacrylonitrile (**12**).

The final stage of the other syntheses was a reaction between 2-thiophenecarboxaldehyde (**9**) and the tetrahydropyrimidine **7**. Although this reaction could be effected by various means, such as heating the reagents in benzene or toluene with azeotropic removal of water, the most efficient process [4] was found to be by use of an equivalent quantity of methyl formate as dehydrating agent (in the absence of a solvent), when a yield of *ca* 40 % of pyrantel (**1**) tartrate was obtained. Use of 2-thiophene-carboxaldehyde-(formyl-^{14}C) [prepared from thiophene (**8**) and dimethylformamide(formyl-^{14}C) in the presence of phosphorus oxychloride [5]] in this final condensation reaction gave pyrantel with the label at the vinylene carbon attached to the thiophene ring.

The radiochemical yields of the four syntheses are listed in Table 1.

From an examination of the location of the labels in pyrantel (Chart I), it can be seen that the hydrolytic cleavage reaction will give rise to labelled thiopheneacrylic acid with two of the labels, and to labelled diamine with the other two labels. Amongst several other possible pathways for metabolic breakdown, a reverse aldol cleavage of the vinylene linkage is possible. The positions of the labels allow information on this point to be obtained, for only the vinylene label will result in a labelled product from the thiophene moiety, the product being either thiophenecarboxylic acid itself or a further breakdown product of the ring system. Furthermore, the fate of the amidine

TABLE 1. Overall Radiochemical Yields for Syntheses of Pyrantel.

Label	Starting material	Final position of label	Radiochemical yield (%)
^{14}C	$K^{14}CN$	C_4-pyrimidyl	19
^{14}C	$CH_3{}^{14}CN$	C_2-pyrimidyl	20
^{14}C	$(CH_3)_2N^{14}CHO$	C_2-vinylene	67[a]
^{3}H	$^{3}H_2$	C_4-pyrimidyl	19[b]

[a] Calculated from 2-thiophene[^{14}C]carboxaldehyde as starting material.
[b] Calculated from N-methyl-1,3-propanediamine-3-^{3}H as starting material.

label can now be used to investigate the metabolic stability of the tetrahydropyrimidine ring. The availability of a tritium label and subsequent determination of tritium water in the animal body after drug treatment provides another probe of the extent of metabolic breakdown experienced by the drug *in vivo*. The results of these investigations will be reported in full detail elsewhere [3].

EXPERIMENTAL.

1. — *N-Benzyl-N-methyl-3-aminopropionitrile-1-^{14}C* (**5**).

A solution of N-benzyl-N-methyl-2-chloroethylamine [6] (**4**) (6.606 g, 36 mmole) in industrial methylated spirit* (10 ml) was added to a stirred, warm solution of KCN (30.5 mCi, i.e. 43.5 mg $K^{14}CN$, sp. act. 45.6 mCi/mmole, plus 2.313 g cold carrier KCN, total 36 mmole) in water (5 ml). The mixture was heated at 100° for 4 hours, cooled to 30°, and the solvent evaporated under reduced pressure. Water (30 ml) was added to the residue, and the mixture was extracted with ether (3 × 50 ml), the extract dried ($CaSO_4$) and the ether evaporated to yield to product as an oil [7], 5.646 g (90.0 %). The chemical purity as determined by gas-liquid chromatography (g.l.c.) (5 % Carbowax 20M on 80/100 mesh Gas-Chrom. Q at 145°) was 99 %.

2. — *N-Benzyl-N-methyl-1,3-propanediamine-3-^{14}C* (**6**).

The nitrile-1-^{14}C **5** (5.646 g, 27.4 mCi) in dry ether (50 ml) was added slowly to a stirred, refluxing mixture of $LiAlH_4$ (7.009 g) in dry ether (250 ml), and the resulting mixture was refluxed with exclusion of water for a further 2.5 hours. The reaction mixture was cooled to 0°, and treated dropwise with water (7 ml) and then sodium hydroxide (5 N, 7 ml) over a period of 40 minutes.

* British Petroleum (U. K.) Ltd., essentially ethanol/water (*ca* 98.5 : 1.5).

The solid was filtered, washed thoroughly with ether, and the combined filtrate and washings evaporated to give the oily product, 4.94 g (85.5 %). The chemical purity (g.l.c., conditions as in experiment 1) was 99 %.

3. — *N-Methyl-1,3-propanediamine-3-^{14}C* (**3**).

The N-benzyl diamine-3-^{14}C **6** (4.94 g, 23.4 mCi) from experiment 2 was dissolved in methanol (75 ml) and hydrochloric acid (*d* 1.18, 9 ml) and hydrogenated over 10 % palladium/charcoal at 60° and a pressure of 2.7 atm. for 18 hours. The catalyst was filtered, and the product obtained as the dihydrochloride salt by evaporation. Conversion to the free base was effected by adding the hydrochloride to saturated KOH solution (35 ml) and extracting with ether (4 × 40 ml). Evaporation of the extract at low temperature yielded the free diamine, 2.009 g (82.4 %). Chemical purity (g.l.c.) was > 99 %.

4. — *1,2-Dimethyl-1,4,5,6-tetrahydropyrimidine-4-^{14}C* (**7**).

N-Methyl-1,3-propanediamine-3-^{14}C (**3**) (1.965 g, 22.3 mole, 18.8 mCi), acetonitrile (1.645 g, 40.1 mmole), and phosphorus pentasulphide (72.7 mg) were heated together at 90° under a reflux condenser for 18 hours. At this time g.l.c. analysis indicated that no starting amine was left in the reaction mixture. The crude product was obtained by decantation from the residual tar, and evaporation of excess acetonitrile at low temperature. The residue was used directly in the preparation of pyrantel-4-^{14}C.

5. — *1,2-Dimethyl-1,4,5,6-tetrahydropyrimidine-2-^{14}C* (**7**).

This preparation differed from experiment 4 only in the proportions of reactants; thus acetonitrile-1-^{14}C (410 mg, 10 mmole) was treated with N-methyl-1,3-propanediamine (810 mg, 9.2 mmole) until g.l.c. showed that no acetonitrile remained in the reaction (40 hours). The yield of crude product was 69.3 %.

6. — *Pyrantel-^{14}C Tartrate from 1,2-Dimethyl-1,4,5,6-tetrahydropyrimidine-^{14}C.*

The crude tetrahydropyrimidine-4-^{14}C (**7**) (2.46 g, 22 mmole, 18.5 mCi) was stirred at 25° with 2-thiophenecarboxaldehyde (2.48 g, 22.1 mmole) and methyl formate (1.38 g, 23 mmole) for 66 hours. Volatile material was removed at room temperature, and the residue treated with tartaric acid (3.30 g, 22 mmole) in 2-propanol/methanol (1 : 1, 15 ml). On cooling at 0° for several hours, the product crystallised and was collected by filtration, and finally washed with 2-propanol/methanol (4 : 1, 2 × 10 ml). The product was recrystallised from 2-propanol/water (9 : 1, 100 ml) with cooling overnight

at 0°. Yield : 2.67 g (7.5 mmole, 30.2 % from N-methyl-1,3-propanediamine-3-^{14}C), specific activity 2.23 μCi/mg, m.p. 144-147°.

A similar preparation starting with 1,2-dimethyl-1,4,5,6-tetrahydropyrimidine-2-^{14}C yielded pyrantel-^{14}C tartrate (labelled in the amidine function) with a specific activity of 1.18 μCi/mg.

7. — *Pyrantel (2-vinylene-^{14}C) Citrate.*

A mixture of 2-thiophenecarboxaldehyde (**9**) (1.0 g, 25 mCi) and 2.376 g unlabelled carrier 2-thiophenecarboxaldehyde (total aldehyde 30.1 mmole) was refluxed in benzene (10 ml) in the presence of 1,2-dimethyltetrahydropyrimidine (**7**) (5.04 g, 45.0 mmole) with a Dean-Stark water-removal tube for a total of 40.5 hours. The contents of the flask were cooled and chromatographed over a column of Woelm Neutral Alumina (80 g) prepared in benzene. The column was eluted with 50 ml fractions of benzene and appropriate fractions containing radioactivity were combined, concentrated to dryness *in vacuo* and the residue was dissolved in ethanol (25 ml). To the pyrantel free base solution was added a solution of citric acid (5.78 g, 30.1 mmole) in ethanol (25 ml), and the product allowed to crystallise by standing at room temperature for several hours. Yield : 8.031 g, 67.0 %, specific activity 1.67 μCi/mg.

8. — *Pyrantel (2-vinylene-^{14}C) Tartrate.*

Pyrantel-^{14}C citrate (2.678 g, 6.72 mmole) was dissolved in 7 ml of water containing KOH (7 g), which had been precooled to 3° C, and then rapidly extracted with 50 ml portions of ether. The ether extracts were combined, dried (Na_2SO_4), concentrated to dryness *in vacuo*, and the residue dissolved in ethanol (10 ml). To this was added tartaric acid (1.008 g, 6.72 mmole) in ethanol (10 ml) and the product allowed to crystallise at room temperature. Yield : 1.793 g, specific activity 1.88 μCi/mg.

9. — *Pyrantel (4,4-pyrimidyl-$^3H_2$) Citrate.*

The iminoether of 2-thiopheneacrylonitrile (**12**) was prepared by bubbling dry HCl gas through a mixture of 2-thiopheneacrylonitrile (**11**) (50 g, 0.37 mole) and anhydrous methanol (15 ml, 0.37 mole) which was cooled by an ice bath. After standing overnight at 0° C the iminoether hydrochloride (37.963 g) was recovered by filtration. A mixture of 2-thiopheneacrylonitrile iminoether hydrochloride (**12**) (2.157 g, 10.6 mmole), N-methyl-1,3-propanediamine-3-^{3}H (**3**) (0.836 g, 9.5 mmole, 1 Ci, prepared by New England Nuclear Corp. by the platinium black reduction of N-methyl-3-aminopropionitrile in the presence of tritium gas) and anhydrous methanol (10 ml), was refluxed for 18 hours with the exclusion of light and moisture. All subsequent extraction

procedures were conducted with solvents cooled to approximately 5° C and performed as rapidly as possible. The reaction mixture was cooled, NaOH (2 N, 75 ml) added, and extracted with several portions of methylene chloride. The methylene chloride was in turn extracted with several portions of HCl (1 N) and this extract basified by the addition of NaOH solution and once again extracted with methylene chloride. To the residue remaining after the removal of methylene chloride was added pyrantel free base derived from the decomposition of unlabelled pyrantel tartrate (7.95 g) as described in Preparation 7 above, and the resulting mixture chromatographed and converted to the citrate salt as described in Preparation 7. Yield : pyrantel-^{3}H citrate, 9.046 g, specific activity 25.8 μCi/mg.

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