NOTES

TRITIUM LABELLING OF CARCINOGENIC CYCLOPENTA[a]PHENANTHRENES AT VERY HIGH SPECIFIC ACTIVITY

SUMMARY

Platinum metal catalysed exchange in [^{3}H]acetic acid is a satisfactory method for generally labelling cyclopenta[a]-phenanthrenes at very high specific activity.

Key words: cyclopenta[a]phenanthrenes, tritium exchange, ^{3}H-n.m.r.

Correct positional methyl substitution in 15,16-dihydro-cyclopenta[a]-phenanthren-17-one (Ia) gives rise to homologues, some of which are carcinogens (12). In order to facilitate studies on metabolism which is involved in their biological activation, several have been labelled with ^{14}C and ^{3}H. Thus [15,16-^{14}C]ketones were obtained using diethyl [2,3-^{14}C]-succinate in Stobbe reactions (3), while [^{3}H- and ^{14}C-methyl]derivatives of Ib were prepared by reaction of the oxoketal II with the appropriately labelled Grignard reagent (3,4). Reduction of II with $NaB^{3}H_{4}$ followed by acid-catalysed dehydration and dehydrogenation gave [11-^{3}H]Ia (5).

Projected experiments on the interaction of these compounds with DNA *in vivo* required activities in excess of 1 Ci/mmol. This could be achieved conveniently using tritium gas, but suitable aryl bromo derivatives which could act as substrates for one-step reductive tritiation were unavailable because electrophilic bromination of Ia or Ib yielded exclusively 15-bromo-(III) and 15,15-dibromo-ketones (IV) (6).

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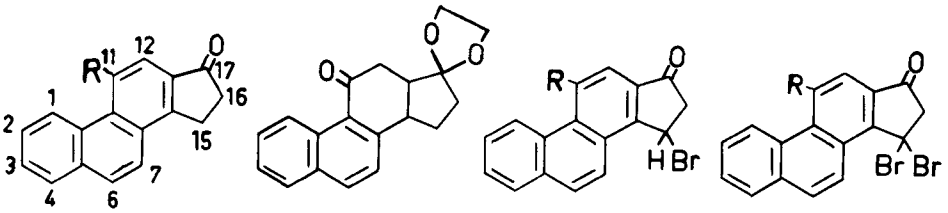


Ia, R=H b, R=CH_3 II IIIa, R=H b, R=CH_3 IVa, R=H b, R=CH_3

Reduction of IIIa dissolved in methanol with palladium-on-calcium carbonate catalyst in tritium/hydrogen gas at normal temperature and pressure for 18 hr. readily gave [15-^{3}H]Ia with the required specific activity (5.2 Ci/mmol) in better than 90% chemical yield. Specificity of labelling was investigated by rebromination to IVa which resulted in loss of 94% of the tritium. Similar reduction of IIIb afforded [15-^{3}H]Ib, 2.2 Ci/mmol; in this case rebromination led to loss of 85% of the isotope, indicating that some 15% was at positions other than C-15. However, these [15-^{3}H]-ketones were not ideal for in vivo experiments because hydroxylation at C-15 is an important metabolic pathway in this series. This results in loss of half the tritium at this position (7), presumably as tritiated water which is subsequently incorporated into biological macromolecules, including DNA. It was hoped that this problem would be lessened by employing generally labelled compounds.

Platinum metal catalysed exchange in acetic acid (8) was carried out on the non-carcinogenic parent ketone Ia as follows:- the ketone (100 mg) was heated in a sealed tube with reduced Adams' catalyst in 70% [^{3}H]acetic acid (1 ml containing approx. 400 Ci) at 150^{o}C for 16 hr. After removal of readily labile tritium by equilibration with ethanol, the product was purified by column chromatography on silica gel, eluting with dichloromethane to give [G-^{3}H]Ia, specific activity 5.81 Ci/mmol, in about 60% chemical yield. Purity was established by ultraviolet light spectroscopy (λ max 265, 284, 296, 334, 359, 367 nm) (3), thin layer chromatography (single

spot, R_F 0.87 on air-dried silica gel plates eluted with toluene, ethyl acetate, methanol (15:5:1 by volume), and high pressure liquid chromatography (single, coincident, radioactive and ultraviolet light absorbing peak eluted at 76 minutes from a Whatman Partisil M9 10/50 ODS column with a gradient of initially 15% methanol in water changing to methanol alone at 1%/minute, flow rate 120 ml/hour). This material was submitted to vigorous acid treatment in order to remove acid-labile tritium. The [G-^{3}H]ketone was heated under reflux with acetic acid-conc. HCl (270 ml, 4:1 ^{v}/v), 250 ml was distilled off, and the whole process was repeated three times more; little tritium was found in the third and fourth distillates. The final, rather dark solution was diluted with water, the mixture was extracted with dichloromethane, and chromatographed as before. The product, designated [aryl-^{3}H]Ia, had specific activity 3.12 Ci/mmol; bromination to IVa reduced this to 2.48 Ci/mmol. Thus acid removes 46% of the original tritium, but about 12% still remains at C-15.

The position of labelling was investigated in detail in the carcinogen [G-^{3}H]Ib which was obtained by the exchange procedure as described (8). After purification the [G-^{3}H]ketone (λmax 264, 288, 301, 342, 358, 370 nm (3); R_F 0.90 (7); elution time 80 minutes) had specific activity 18.3 Ci/mmol; treatment of a portion with acid gave the [aryl-^{3}H] ketone at 13.9 Ci/mmol, showing a loss of 24% acid-labile tritium. Samples of these ketones in $CDCl_3$ were examined by tritium n.m.r. spectroscopy (8) as shown in the table and figure. As expected acid removed tritium alpha to the ketone quantitatively, and tritium at benzylic hydrogen positions much less effectively. Bromination of [G-^{3}H]Ib to [G-^{3}H]IVb resulted in loss of 29% of the tritium, in reasonable agreement with that expected (19 + 14.8 = 33.8%) from the n.m.r. data. Evidently electrophilic bromination removes tritium from C-16, as well as C-15, owing to the acid conditions generated by the liberation of HBr.

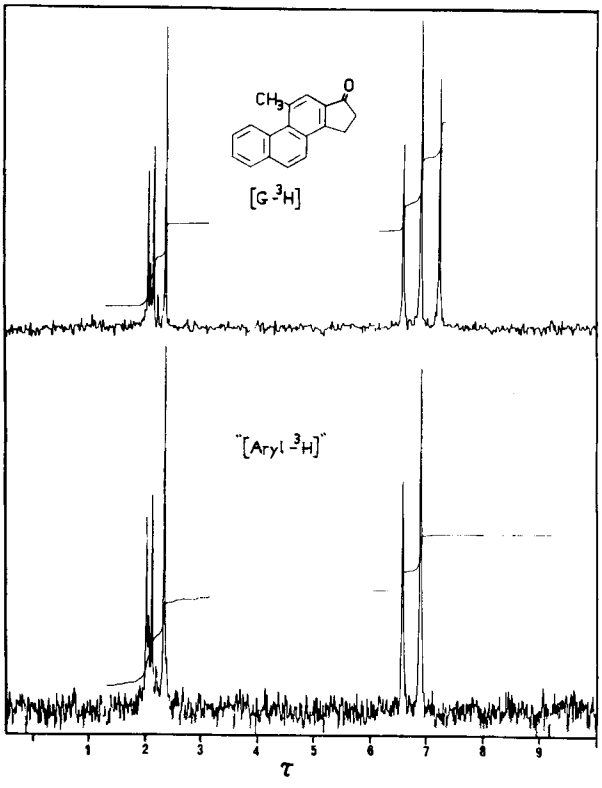
TRITIUM n.m.r. SPECTRA OF [G-^{3}H]Ib AND [aryl-^{3}H]Ib

Line position				Relative labelling		
δ_H [a]	δ_T [b]	τ	Assignment	[G-^{3}H]	"[aryl-^{3}H]" [c]	Difference
2.84	2.84	7.16	16-CH_2	19.0	0.0	-19.0
3.17	3.17	6.83	11-CH_3	23.1	19.4	-3.7
3.46	3.46	6.54	15-CH_2	14.8	12.5	-2.3
7.67	7.72	2.28	2,3-H	17.4	17.9	+0.5
7.79	7.86	2.14	12-H	1.9	2.7	+0.8
7.90	7.93	2.07	6-H	8.7	6.2	-2.5
	7.99	2.01	7-H	3.2	3.4	+0.2
7.98	8.03	1.97	4-H	11.9	13.8	+1.9
8.95	-	-	1-H	0.0	0.0	-

[a] From TMS at 90 MHz (unlabelled sample)

[b] From γ_{TMS} x 1.06663975 at 96 MHz

[c] Allowing for the observed loss of 24% of tritium



It is interesting that all positions are labelled in the [G-^{3}H]ketone with the exception of C-1, which is presumably sterically hindered by the 11-methyl group from approaching the catalyst surface. Also labelling at the partially hindered C-7 and C-12 positions is lower than that at the unhindered C-2, -3, -4, and 6- positions; the 11-methyl group is particularly heavily labelled. This pattern is similar to that found for 3-methylcholanthrene and 7-methylbenz[a]anthracene labelled by this procedure (8).

This method of preparing "[aryl-^{3}H]"-ketones has now been applied to five compounds (Ia, Ib, and 6-, 7- and 12-methylIa); specific activities fall in the range 3-30 Ci/mmol. These compounds are now being used in biological experiments, the results of which will be published elsewhere.

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