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The Simple Preparation of Mevalonic-3', 4-D Acid and -3', 4-T Acid

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SUMMARY

Mevalonic-3′, 4-D acid and —3′, 4-T acid were synthesized by using deuterated and tritiated water and the total yield of the labelled MVA was 6.5 %. The specific activity of the tritiated MVA, which was derived from 2.3 g. of tritiated water (250 mCi), was 1.77 mCi./mM.

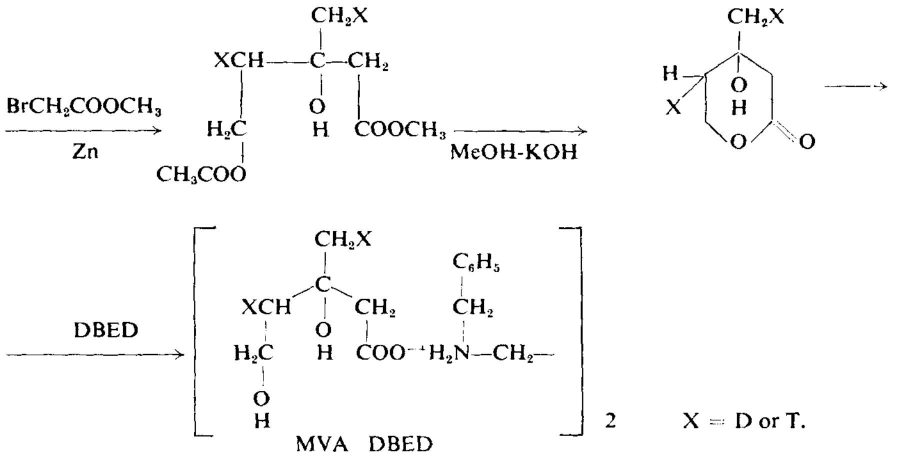
INTRODUCTION.

Since mevalonic acid (MVA) is an important biosynthetic intermediate of steroids and terpenoids in animals, plants and microorganisms, labelled MVA has often been used for the biochemical studies. Although the several labelled MVAs with deuterium, tritium and radioactive carbon have been synthesized and some of them are available commercially, they are somewhat expensive and thus the use of labelled MVA is limited.

This paper describes the preparation of MVA-3′, 4-D and -3′, 4-T. These preparations have not as yet been reported. They are simply and cheaply synthesized by using deuterated and tritiated water.

The addition of acetic acid-D or -T, prepared from acetic anhydride and deuterated or tritiated water, to methyl vinyl ketone gave 3-oxobutyl-2,4-D or-T acetate, which was subsequently converted to methyl 3-hydroxyl-3-methyl-5-acetoxypentanoate-3′,4-D or -T by treatment with methyl bromoacetate in the presence of zinc. The ester was hydrolyzed with 1 N methanolic potassium hydroxide to afford MVA-3′,4-D or -T. The labelled MVA was purified as the dibenzyl ethylenediamine (DBED) salt. The synthesis of the labelled MVA was carried out as follows;

$$(CH_3CO)_2O \xrightarrow{X_2O} CH_3COOX \xrightarrow{CH_3COCH = CH_2} CH_2XCOCHXCH_2OCOCH_3 \longrightarrow$$



EXPERIMENTAL.

Analysis of Deuterium and Tritium.

Deuterium was analyzed by NMR spectrometry using a Varian HR-100. The radioactivity of tritium was determined using a Tri-Carb liquid scintillation spectrometer 314 EX.

3-*Oxobutyl-*2, 4-*D acetate (I)* 1).

The mixture of methyl vinyl ketone (11 g., bp. 77-85° C) and acetic acid-D (30.5 g), which was prepared from acetic anhydride (25.5 g) and deuterated water (5 g), was refluxed gently for 17 hr. The fractional distillation of the reaction mixture under reduced pressure afforded I (7.3 g, bp$_{15}$.78-85° C).

Methyl 3-*hydroxyl*-3-*methyl*-5-*acetoxypentanoate-3′*, 4-*D* (II) 1).

I (3 g) and methyl bromoacetate (3.5 g) were dissolved in abs. ether (15 mg). Mossy zinc (3 g) and a small amount of iodine was added to the mixture. The reaction mixture was refluxed for 4 hr with stirring and lots of gummy complex was separated from the solution. After cooling, acetic acid (1.3 ml) and water (10 ml) were added to the reaction mixture. The ether layer was removed and the water layer was saturated with ammonium chloride. It was extracted four times with ether. The ether extract was washed with saturated sodium bicarbonate solution (15 ml) and dried over anhyd. sodium sulphate. After removal of ether, the residue was distilled under reduced pressure to afford II (1.55 g, bp$_{0.37}$.60-100° C).

Mevalonic-3′, 4-D acid dibenzyl ethylenediamine salt (III).

II (1.55 g) was dissolved in 1 N methanolic potassium hydroxide (30 ml) and allowed to stand for 48 hr at room temperature. The reaction mixture was neutralized by passing in dried hydrogen chloride gas. Congo red was used as an indicator. The precipitate of potassium chloride was filtered off. Methanol was removed in vacuo from the filtrate. The residue was extracted with chloroform. The chloroform was removed in vacuo and the residue was distilled under reduced pressure to afford MVA-3′, 4-D lactone (IV) (587 mg, $bp_{0.25}$.90-120° C).

IV (130 mg) and DBED (200 mg) were dissolved in a mixture of methanol (2 ml) and water (1.5 ml), and the mixture was allowed to stand overnight at room temperature. After methanol was distilled off, the water layer was extracted with chloroform to remove the impurities. Water from the solution was removed to give the crude III. The recrystallization of the crude III from the mixture of methanol and ether gave pure III (200 mg., mp 123° C).

Anal. Calcd. for $C_{28}H_{44}N_2O_8$: C, 62.65; H, 8.26; N, 5.23.
Found : C, 62.69; H, 8.15; N, 5.05.
(a non-isotopic specimen prepared in the same way, was analysed).

Mevalonic-3′, 4-T acid dibenzyl ethylenediamine salt (V).

V was synthesized by the same method as described above from methyl vinyl ketone (6 g) and acetic acid-T (15 g, 250 mCi), which was prepared from acetic anhydride (12.7 g) and tritiated water (2.3 g, 250 mCi). The yield of V was 1.5 g (yield; 6.5 % based on methyl vinyl ketone, specific acitivity 1.77 mCi/mM).

DISCUSSION.

The NMR data of 3-oxobutyl acetate, I, MVA DBED and III were shown in Table I and II.

The NMR spectrum of 3-oxobutyl acetate exhibited a singlet at 2.20 ppm. for CH_3COC and a triplet at 2.80 ppm.′ for $COCH_2C$. On the other hand the intensity of a singlet at 2.20 ppm. and a triplet at 2.80 ppm. was decreased and the shape of a signal at 4.30 ppm. for CCH_2O was deformed. These observations suggest that some of the hydrogens attached to C-2 and C-4 of 3-oxobutyl acetate were displaced by deuterium. Therefore it appears that in the synthesis of I, acetic acid-D added to the double bond of methyl vinyl ketone and deuterium exchange reaction occurred at the methyl group of methyl ketone in methyl vinyl ketone and/or 3 oxobutyl acetate.

The NMR spectrum of the deuterated MVA, prepared from I, showed that some of the hydrogens attached to C-3′ and C-4 were displaced by deu-

TABLE I. The NMR data of 3-oxobutyl acetate and the deuterium analysis in the molecule of 3-oxobutyl-2,4-D acetate.

	3-Oxobutyl acetate		3-Oxobutyl-2,4-D acetate		
Position of hydrogen	Chemical shift (δ)	Intensity	Intensity	Decrease of intensity by D displacement	Ratio of D in the molecule (%)
CH_3COOC	2.01 (s)	3.0	3.0	0	0
CCH_2O	4.30 (t)	2.0	2.0	0	0
$COCH_2C$	2.80 (t)	2.0	1.5	0.5	36
$CCOCH_3$	2.20 (s)	3.0	2.1	0.9	64
$CH_3COCH_2CH_2OCOCH_3$		10.0	8.6	1.4	100

TABLE II. The NMR data of MVA DBED and the deuterium analysis in the molecule of MVA-3′, 4-D DBED.

	MVA DBED		MVA-3′, 4-D DBED		
Position of hydrogen	Chemical shift (δ)	Intensity	Intensity	Decrease of intensity by D displacement	Ratio of D in the molecule (%)
CCH_2COO	2.33 (s)	2.0	2.0	0	0
CH_3C	1.23 (s)	3.0	2.1	0.9	64
CCH_2C	1.80 (t)	2.0	1.5	0.5	36
CCH_2OH	3.70 (t)	2.0	2.0	0	0
C_6H_5C	7.47 (s)	5.0	5.0	0	0
Ph-CH_2N	4.23 (s)	2.0	2.0	0	0
NCH_2C	3.40 (s)	2.0	2.0	0	0
MVA DBED		18.0	16.6	1.4	100

terium in the same manner as I, and the deuterium in the molecule was 64 % at C-3′ and 36 % at C-4.

Though the labelling pattern of the tritiated MVA has not been determined by a degradation method, the labelling pattern should be equivalent to that of the deuterated MVA synthesized by the same method.

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