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SYNTHESIS OF RADIOACTIVE ZEATIN RIBOSIDE AND RELATED COMPOUNDS BY ALKYLATION OF PURINE MOIETIES

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Key Word Index—(*E*)-4-Hydroxy-3-methylbut-2-enyl moiety; zeatin; zeatin riboside; cytokinins; synthesis.

Abstract—A method for the synthesis of (*E*)-4-(tetrahydropyran-2-yloxy)-3-methylbut-2-enyl bromide is described and its use in the synthesis of [^{3}H]zeatin riboside, zeatin and a xanthine derivative by alkylation is reported.

INTRODUCTION

The (*E*)-4-hydroxy-3-methylbut-2-enyl moiety is a key structural feature of zeatin and numerous related purine compounds with cytokinin activity [1]. In synthesis of such compounds, the availability of (*E*)-4-(tetrahydropyran-2-yloxy)-3-methylbut-2-enyl bromide would often be advantageous as it could be used in alkylation reactions to introduce the above hydroxybutenyl moiety into purine derivatives. The synthesis of this bromide is described herein and its use in the synthesis of zeatin and related compounds including [^{3}H]zeatin riboside is reported.

RESULTS AND DISCUSSION

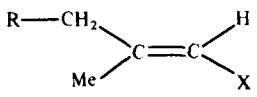
Mild hydrolysis of methyl 4-bromo-3-methylcrotonate (*cis–trans* mixture) readily yielded pure (*E*)-methyl 4-hydroxy-3-methylcrotonate (**1**) because the *cis*-hydroxy ester underwent facile lactonization. Conversion of **1** to the tetrahydropyranyl ether (**2**) followed by reduction with lithium aluminium hydride yielded the alcohol **3** which was converted to the corresponding bromide (**4**) and chloride by reaction with tetrabromo- and tetrachloromethane, respectively, in the presence of trioctyl phosphine. The allylic bromide **4** was unstable, but it was used to alkylate [^{3}H]adenosine, 9-(tetrahydropyran-2-yl)adenine and 1,7-dimethylxanthine to give [^{3}H]zeatin riboside, zeatin and (*E*)-3-(4-hydroxy-3-methylbut-2-enyl)-1,7-dimethylxanthine, respectively, after cleavage of the tetrahydropyranyl moiety by acid hydrolysis. Hence, **4** may serve as a useful reagent for introduction of the (*E*)-4-hydroxy-3-methylbut-2-enyl moiety by alkylation. The above xanthine derivative was one of a series of such compounds synthesized as potential inhibitors of cytokinin metabolism.

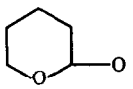
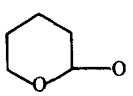
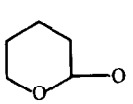
By the above method, [^{3}H]zeatin riboside of high specific activity was synthesized. Because only the final synthetic step involves a radioactive compound (adenosine) and as any unreacted adenosine can be readily recovered chromatographically, the procedure minimizes losses of radioactive precursor. In this respect, it has advantages over the only other reported synthesis of zeatin riboside of high specific radioactivity [2]. When 9-(tetrahydropyran)-2-yl-adenine was converted to its anionic form with NaOEt [3] and then alkylated with **4**, zeatin was obtained in good yield after cleavage of pyranyl groups with acid. This unambiguous synthesis yielded the zeatin which was exchanged catalytically with ^3H_2O [4] and was used in initial studies of the metabolism and translocation of this hormone [5].

EXPERIMENTAL

Chromatographic methods. For TLC, layers spread with Merck silica gel 60 PF_{254} or cellulose [6] were employed. The following solvents were used: A, *n*-BuOH–14 M NH_4OH–H_2O (6:1:2, upper phase); B, $CHCl_3$–MeOH (9:1).

Methyl 4-bromo-3-methylcrotonate. A soln of Me 3,3-dimethylacrylate (25 g) in CCl_4 (46 ml) was heated under reflux (incan-



	R	X
1	OH	COOMe
2		COOMe
3		CH_2OH
4		CH_2Br

descent lamp) with *N*-bromosuccinimide (39.4 g) until conversion to succinimide was complete (*ca* 2 hr). The mixt. was then left at 4°, the succinimide filtered off and the CCl_4 evapd. Dist. of the residue yielded the bromoester as a *cis/trans* (1:2) mixt. bp 118–125°/45 mm (cf. ref. 7]. The bromo Et ester (*cis/trans*) was prepared similarly.

(E)-*Methyl* 4-*hydroxy*-3-*methylcrotonate* (**1**). A soln of the above *cis/trans* Me ester (17.5 g) in a mixt of dioxan (66 ml) and H_2O (33 ml) was stirred mechanically with $BaCO_3$ (8.95 g) and heated under reflux for 48 hr. Most of the dioxan was evapd under red. pres. and any material not in soln was filtered off and washed with Et_2O. The filtrate was extd with Et_2O (4× using equal vol.) which was then combined with the Et_2O washings. The resulting soln was dried ($MgSO_4$) and concd; fractional dist. yielded the lactone of 4-hydroxy-3-methylbut-2-enoic acid (derived from the *cis* ester) followed by **1**, bp 93–94°/3 mm. ^{1}H NMR ($CDCl_3$, 60 MHz): δ6.02 (1H, *m*, C=CH), 4.14 (2H, *br s*, CH_2O), 3.70 (3H, *s*, COOMe), 2.42 (1H, *s*, exchangeable D_2O, OH), 2.10 (3H, *d*, $J = 1$ Hz, Me–C=C). Similar hydrolysis of the bromo Et ester with $BaCO_3$ yielded (*E*)-ethyl-4-hydroxy-3-methylcrotonate, bp 100°/0.4 mm. EIMS (probe) 70 eV, *m/z* (rel. int.): 144 $[M]^+$ (17), 126 (13), 115 (30), 99 (71), 98 (100), 87 (32), 71 (43) and 69 (32).

(E)-*Methyl* 4-(*tetrahydropyran*-2-*yloxy*)-3-*methylcrotonate* (**2**). Trifluoroacetic acid (10 μl) was added to a soln of hydroxy Me ester (**1**) (14 g) in dry 2,3-dihydropyran (10.2 g, dist. from $LiAlH_4$). The soln became warm after 10 min and was left for 48 hr when the products were fractionated at 50°/10^{-3} mm using a short path dist apparatus. The dist contained impurities and the residue was **2**. ^{1}H NMR ($CDCl_3$, 60 MHz): δ6.0 (1H, *m*, C=CH), 4.65 (1H, O–CH–O), 4.0–4.20 (2H, *m*, O–CH_2–C=C), 3.50–3.90 (5H, *m*, –COOMe singlet plus ring CH_2–O), 2.12 (3H, *d*, $J = 1$ Hz, Me–C=C), 1.68 (6H, *br m*, ring CH_2). The tetrahydropyranyl Et ester was prepd by the same procedure, bp 110–120°/0.7 mm.

(E)-4-(*tetrahydropyran*-2-*yloxy*)-3-*Methylbut*-2-*en*-1-*ol* **3**. The above ester **2** (20.2 g) was dissolved in dry Et_2O (100 ml) and the soln added slowly to a stirred suspension of $LiAlH_4$ (2.72 g) in 200 ml of dry Et_2O. After 1 hr H_2O followed by dil NaOH soln was added at 5° to decompose the reduction complex. The filtered Et_2O soln was dried (Na_2SO_4) and evapd. The residue was distilled to yield **3** (90% yield), bp 95°/10^{-3} mm. ^{1}H NMR ($CDCl_3$, 60 MHz): δ5.65 (1H, *br t*, $J = 7$ Hz, C=CH), 4.60 (1H, O–CH–O), 3.87–4.33 (4H, two CH_2O), 3.43–3.83 (2H, *m*, ring CH_2–O), 2.65 (1H, *br s*, exchangeable D_2O, OH), 1.22–1.98 (9H, Me–C=C singlet plus three CH_2 of ring). The ^{13}C NMR spectrum ($CDCl_3$, noise decoupled) revealed 10 C atoms: δ134.8 (C-3), 126.3 (C-2), 97.7 (O–CH–O), 72.1 (–CH_2O–), 62.1 (C-1), 58.6 (–CH_2O–), 30.5 (ring C), 25.5 (ring C), 19.4 (ring C), 14.0 (Me at C-3). In the off resonance decoupled spectrum, signal multiplicity was *s*, *d*, *d*, *t*, *t*, *t*, *t*, *t*, *t* and *q*, respectively. EIMS (probe) 70 eV, *m/z* (rel. int.): 186 $[M]^+$ (0.4), 185 (1), 169 (7), 168 (5), 156 (2), 101 (23), 85 (100) and 67 (65). Reduction of the Et ester by the same procedure yielded **3** in 91% yield.

(E)-4-(*tetrahydropyran*-2-*yloxy*)-3-*Methylbut*-2-*enyl bromide* **4**. Trioctylphosphine (2.13 g) was added dropwise to a stirred soln of the alcohol **3** (1 g) and CBr_4 (2.13 g) in dry C_6H_6–petrol (1:4, 15 ml) cooled to −20° and maintained under dry N_2. The stirring was then continued for a further 45 min at −20° when the petrol layer was removed. The residue was washed with petrol (2 × 2 ml) and the combined petrol solns evapd to yield the crude bromide **4** (2.1 g) as an oil which is suitable for alkylation reactions (see below). Compound **4** (bp 43–47°/5 × 10^{-4} mm) is unstable and is difficult to purify completely. However, the more stable chloride, prepd by the same method, can be obtained in a state of purity by TLC (silica gel, Et_2O–petrol, 1:9). ^{1}H NMR ($CDCl_3$, 60 MHz): δ5.75 (1H, *br t*, $J = 8$ Hz, C=CH), 4.60 (1H, O–CH–O), 3.35–4.30 (6H, *br m*, –CH_2Cl + O–CH_2–C=C + ring CH_2O), 1.33–2.13 (9H, CH_3–C=C singlet + three CH_2 of ring).

Synthesis of [^{3}H]*zeatin riboside*. [2-^{3}H] Adenosine (24.0 Ci/mmol, 80 μg), adenosine (200 μg) and the crude bromide **4** (15 μl) were dissolved in dry DMF (0.2 ml) in the presence of four 4A molecular sieves. The soln was then left to stand for 3 days at room temp. in the dark. Solvent was evapd under N_2 and the residue taken up in Me_2NH–MeOH (1:1) and allowed to stand at room temp. for 6 hr to rearrange the 1-substituted adenosine formed to the N^6 isomer. Solvent was then removed under N_2. The tetrahydropyranyl group was hydrolysed in 0.1 M HCl at 24° for 24 hr. The pH of the soln was adjusted to 8–9 with NaOH and the [^{3}H]ZR extd with H_2O-satd *n*-BuOH and purified by TLC first on silica gel and then on cellulose (solvent A). The [^{3}H]ZR was eluted with MeOH–HOAc–H_2O (10:1:10), solvent evapd and the residue redissolved and stored in 50% EtOH at −20°. Yield 60 μg, sp. act. 4.8 Ci/mmol. The identity of the product was confirmed by UV, HPLC, reversed phase TLC [8] and GC-MS.

Synthesis of zeatin. 9-(tetrahydropyran-2-yl)Adenine was converted to its anionic form with NaOEt and then alkylated with **4** under conditions used previously for the synthesis of N^6-substituted adenines [3]. Cleavage of the tetrahydropyranyl groups in 0.2 M HCl yielded zeatin (yield 80%) identified by mp, UV and MS.

Synthesis of (E)-1,7-*dimethyl*-3-(4-*hydroxy*-3-*methylbut*-2-*enyl*)-*xanthine*. 1,7-Dimethylxanthine (35 mg) was dissolved in dry DMF and a 20% excess of NaH was added. The mixt was stirred at 60–70° for 20 min when **4** (45 μl) was added. When TLC on silica gel (solvent B) indicated reaction was complete, the DMF was dild with H_2O and extd with 3 equal vols of EtOAc. The extd fr. was subjected to TLC (silica gel, solvent B) and the UV-absorbing zone at R_f 0.63 eluted ($CHCl_3$–MeOH, 1:1) and hydrolysed in 50% MeOH containing HCl (0.1 M) at 24° for 18 hr to cleave the tetrahydropyranyl moiety. Crystallization from EtOH–H_2O yielded (*E*)-1,7-dimethyl-3-(4-hydroxy-3-methylbut-2-enyl)xanthine (yield 32%), λ_{max} 273 nm in both 70% EtOH and 70% EtOH containing NH_4OH (0.4 N). EIMS (probe) 70 eV, *m/z* (rel. int.): 264 ($[M]^+$, 18), 246 (20), 233 (25), 194 (5), 181 (100), 180 (59), 163 (7), 161 (13), 151 (10), 136 (11) and 123 (60). (Found *m/z*: $[M]^+$ 264.1220, $C_{12}H_{16}N_4O_3$ requires 264.1222).

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