Tetrahedron Letters,Vol.23,No.17,pp 1817-1820,1982 0040-4039/82/171817-04$03.00/0
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SIMPLE, INEXPENSIVE ROUTES TO E AND Z ZEATINE RIBOSIDES AND DERIVATIVES USEFUL FOR IMMUNOASSAY.

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Abstract. Allylic oxidation of 6-N-(3,3-dimethylallyl)adenosine 1 gave trans (E) zeatine riboside 3, which was isomerized to the cis (Z) isomer 5 by u.v. irradiation. A method for the regiospecific 3'-O-succinoylation of these nucleosides is given.

The β-D-ribofuranoside 3 of the so-called "trans" zeatine is a well-known, highly active stimulant of cell divisions in plant tissue cultures,[1] while the related "cis" isomer 5 has been isolated from the t-RNA of certain plant tissues.[2] The reported syntheses[3,4,5] of 3 and 5 are multistep procedures from commercial chemicals. For this reason, we looked for shorter routes when it appeared that antibodies against these structures would be most useful for cytokinine immunoassay.

The reported syntheses of nucleosides 3 and 5 begin with the construction of precursors of the substituent chains which already have the desired configuration, that is either (E)- or (Z)-4-amino-2-methylbut-2-en-1-ol. These amines were then condensed with 6-chloro-9-β-D-ribofuranosylpurine. In our different approach the starting-material was 6-N-(3,3-dimethylallyl)adenosine, 1, which can be prepared in one step from adenosine and "isoprenyl bromide".[6] Allylic oxidation of the isopentenyl substituent with a mixture of ter-butyl hydroperoxide and selenium dioxide[7] gave regiospecifically the (E) isomer 3. Because of decomposition (visible by t.l.c.), the reaction was not allowed to go to completion. Still, "trans" zeatine riboside 3 was finally isolated in 44% yield, so that this cytokinine is now available in two easy steps from inexpensive chemicals.

Isomerization of nucleoside 3 by u.v. irradiation in ethanol, monitored by t.l.c., led to an apparent equilibrium mixture with the "cis" isomer 5. From this mixture, "cis" zeatine β-D-ribofuranoside 5 could be isolated in 38% yield, thus completing an overall three step synthesis.

In order to bind the cytokinines 1, 3 and 5 to bovine serum albumine, while respecting as much as possible their integrity, these were esterified to the corresponding 3'-O-(acid succinates), 2, 4 and 6. Preliminary experiments showed that the known[8] stannylenes of adenosine did not react with succinic anhydride in a variety of conditions. Esterification was possible only in dry pyridine, in the presence of tetrabutylammonium bromide (cf.[9]). By this procedure, adenosine and the three cytokinines 1, 3, 5 gave in a few minutes a quantitative yield of only one ester. This was the 3'-O acid succinate for proton 3'-H was deshielded by 1 p.p.m. relative to the parent nucleoside (Table). The acid succinates 2, 4, 6 were

solids which tenaciously retained traces of inorganic material, but their composition could be ascertained by high resolution mass spectrometry. One important fragmentation was the loss of the succinoyl chain $CO-(CH_2)_2-CO_2$, followed in the case of 4 and 6 by the loss of OH.

Coupling of these carboxylic derivatives to bovine serum albumine by the method of Erlanger et al.[10] gave conjugates with 20 moles of cytokine per mole of protein. Immunisation results will be reported elsewhere.

6-[(E)-4-Hydroxy-3-methylbut-2-enylamino]-9-β-D-ribofuranosylpurine (3).- tert-Butyl hydroperoxide in benzene (90%; 1.7 ml) and SeO_2 (333 mg) were added to a suspension of nucleoside 1 (2.01 g) in CH_2Cl_2 (200 ml). The mixture was vigorously stirred for 24-48 h at 32°C, and then made homogeneous by addition of methanol. Chromatography[11] ($CHCl_3$-methanol, 17:3) of the solution gave the E-nucleoside 3 (930 mg), m.p. 181-2°C (from alcohol) (lit.[3] 180-2°C), $[\alpha]_D^{20}$ -17° (c 1, H_2O).

6-[(Z)-4-Hydroxy-3-methylbut-2-enylamino]-9-β-D-ribofuranosylpurine (5).- A solution of 3 (351 mg) in ethanol (200 ml) was irradiated for 48 h by a high pressure, 450 W Hanovia lamp in a quartz reactor, and then evaporated to dryness. Chromatography[11] of the residue ($CHCl_3$-methanol, 17:3) gave the Z isomer 5 (134 mg), m.p. 201-2°C (from ethanol) (lit.[4] 202-5°C).

General procedure for the preparation of acid succinates.- A methanol solution (15 ml) of the dibutylstannylene of the nucleoside (1 mmole) was prepared in the usual way[8] and then evaporated to dryness. The residue was dissolved in dry pyridine (20 ml), succinic anhydride (1.1 mmole) and tetrabutylammonium bromide (1 mmole) were added. After a few minutes, the mixture was evaporated to dryness.

3'-O-Succinoyladenosine.- A chloroformic solution (25 ml) of the residue was shaken with Dowex 50 W resin (X2, 50-100 mesh, Na^+) and water (25 ml). Evaporation to dryness of the aqueous phase and chromatography[11] of the residue (ethyl acetate-2-propanol-water, 3:3:1) gave the half ester (93%), m.p. 181-5°C (from acidified water); acceptable analysis (C,H,N,O) for $C_{14}H_{17}O_7N_5$, H_2O.

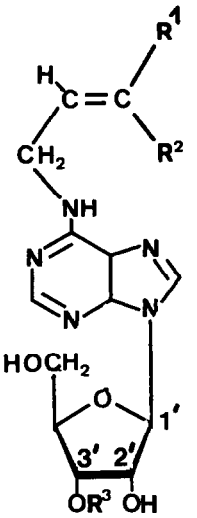
Mass spectrometric data.- The following esters were purified by chromatography[11] (chloroform-methanol, 17:3). Abbreviations : FDCI field desorption-chemical ionization (NH_3); HREI high resolution electron impact spectroscopy.

Ester 2.- FDCI . m/e 436 (15%) (MH^+); 336 (100%) $[(MH-C_4H_4O_3)^+]$. HREI : m/e 335.1597 (48%) $[(M-C_4H_4O_3)^+]$; calculated for $C_{15}H_{21}N_5O_4$: 335.1589.

Ester 4.- FDCI : m/e 452 (2%) (MH^+), 352 (100%) $[(MH-C_4H_4O_3)^+]$. HREI : m/e 334.1514 (43%) $[(M-C_4H_4O_3-OH)^+]$; calculated for $C_{15}H_{20}N_5O_4$: 334.1511.

Ester 6.- FDCI · m/e 452 (6%) (MH^+), 352 (100%) $[(MH-C_4H_4O_3)^+]$. HREI : m/e 320.1348 $[(M-C_4H_4O_3-CH_2OH)^+]$; calculated for $C_{14}H_{18}N_5O_4$ 320.1355.

Acknowledgments.- The authors are indebted to Dr R. Beugelmans for his help in the u.v. experiments.



	R^1	R^2	R^3
1	Me	Me	H
2	Me	Me	$-COCH_2CH_2COOH$
3	$-CH_2OH$	Me	H
4	$-CH_2OH$	Me	$-COCH_2CH_2-COOH$
5	Me	$-CH_2OH$	H
6	Me	$-CH_2OH$	$-COCH_2CH_2-COOH$

Table · Selected NMR chemical shifts.[a]

	1'-H	2'-H[d]	3'-H	Me
Adenosine[b]	5.97 (6)	4.67 (6)	4.25	
1[c]	5.84 (6)	4.60 (5)	4.16 (3.5)	1.67; 1.70
3[b]	5.88 (6)	4.59 (5.4)	4.20	1.66
5[c]	5.91 (6)	4.60 (5.5)	4.20	1.74
3'-O-Succinyl-adenosine[b]	5.90 (6)	4.90 (5)	5 30 (1.5)	
2[a]	5 90 (6)	4.88 (5)	5.30	1.67, 1.70
4[b]	5.93 (6.8)	4.86 (5)	5.28 (2)	1.68
6[c]	5.90 (6)	4.84 (6)	5.28	1.72

[a] In p.p.m. downfield from Me_4Si, for solutions in mixtures of dimethylsulfoxyde-d_6 and D_2O; the coupling constants in brackets are $J_{n,n+1}$; all the attributions have been checked by double irradiation; [b] at 90 MHz; [c] at 250 MHz, 67°C; [d] acetylation brought about a 1 p.p.m. downfield shift.

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11. Silica gel column chromatography. The same eluent was used for t.l.c. control of the reaction.

(Received in France 20 January 1982)