SYNTHESIS OF 6-(3-METHYLPYRROL-1-YL)-9-ß-D-RIBOFURANOSYL PURINE, A NOVEL METABOLITE OF ZEATIN RIBOSIDE

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Summary: The allylic alcohol in ribose-protected zeatin riboside **1**, was oxidised to an aldehyde by barium manganate. Heating of the resulting aldehyde in chloroform allowed the cyclization of the N^6-chain into a 3-methylpyrrole ring. After deprotection of the ribose, the title compound was obtained and identified to a new metabolite of zeatin riboside. Its aglycone was synthesized through a similar route from N-9 protected zeatin.

Cytokinins constitute a class of phytohormones which stimulate the division of plant cells and are involved in many aspects of the development of plants. During the investigation of the biosynthesis of cytokinins in tobacco crown gall cells, we isolated[1] a new metabolite of zeatin riboside. Its mass spectrum showed a 20 units mass loss from the parent compound **1** and indicated that the ribosyl moiety was still present. The ribose could be eliminated by acidic treatment (HCl 0.1 M, 100°C, 1 h), as confirmed by mass spectrometry. Furthermore, the uv spectra of the metabolite did not change markedly with pH, as observed in N^6,9-disubstituted adenines,[2] and the uv spectra of the expected aglycone resulting from its acid hydrolysis behaved like those of N^6-mono or disubstituted adenines.[3] However, all the uv spectra of the metabolite and its aglycone were bathochromically shifted of about 20 nm from the spectra of the parent compounds zeatin riboside **1** or zeatin **2** respectively.

1 R = ß-D-ribofuranosyl
2 R = H
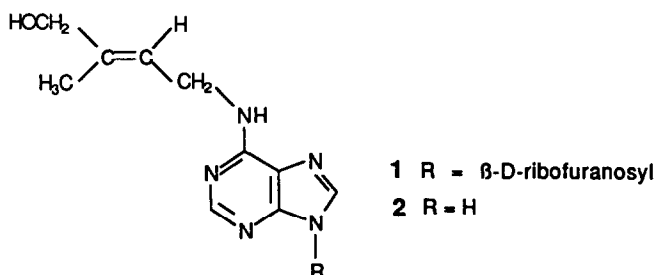
On this basis, we postulated that the metabolite could be 6-(3-methylpyrrol-1-yl)-9-ß-D-ribofuranosyl-purine **3**. This was confirmed by its chemical synthesis which we describe herein (Scheme 1), with the synthesis of its aglycone (Scheme 2).

The zeatin riboside derivative **4** was prepared from [(E)-4-hydroxy-3-methylbut-2-enyl]-ammonium sulphate and chloropurine riboside whose ribose OH groups were protected by the t-butyldimethylsilyl group.[4,5] Then, the allylic alcohol was oxidized with baryum manganate[6] in dichloromethane in which compound **4** was readily soluble.
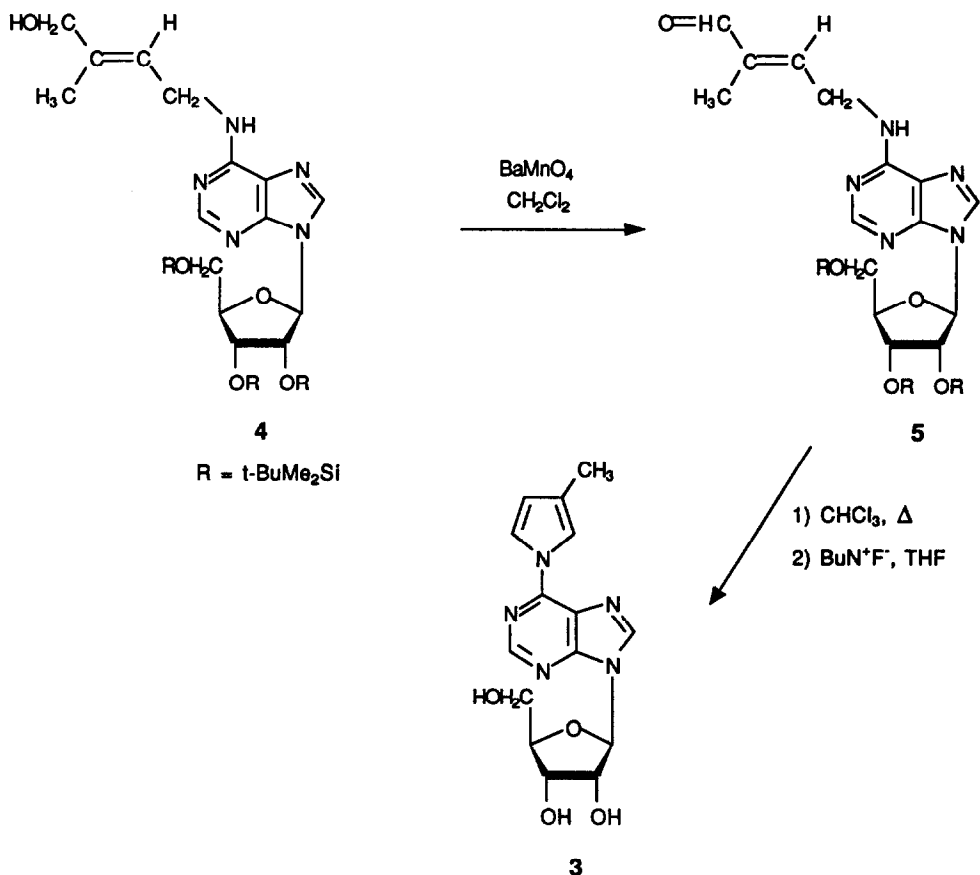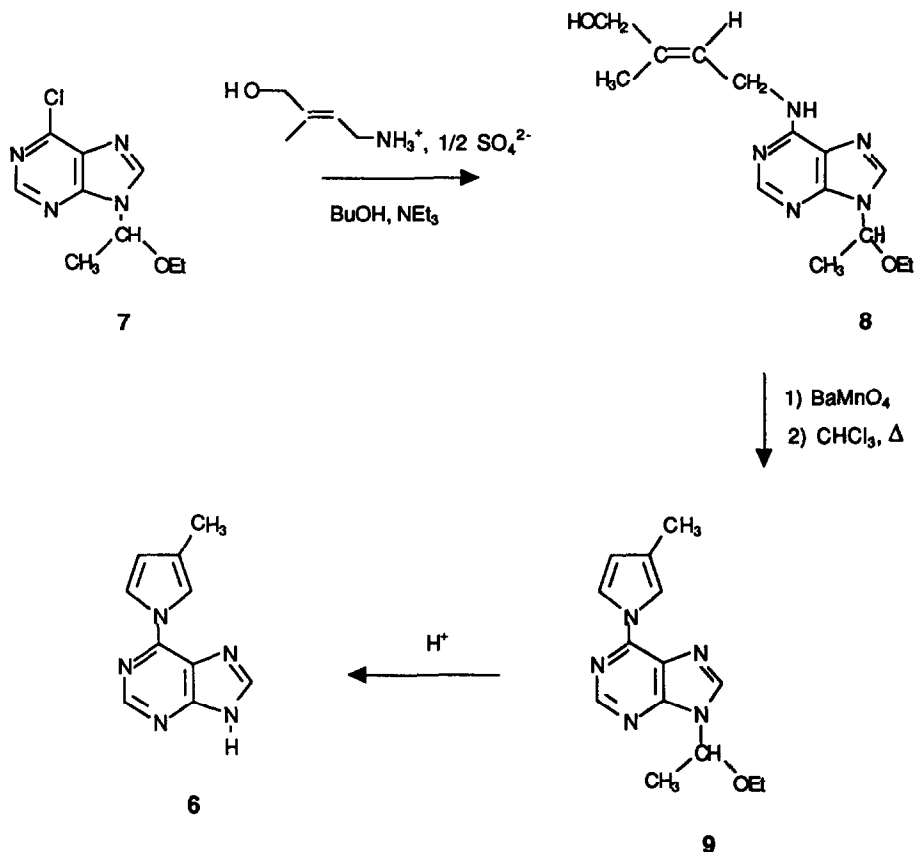

Scheme 1

The smooth reaction conditions (room temperature, 48 h stirring) allowed us to isolate and characterize the aldehyde[7] **5** which was cyclized by refluxing in chloroform (40 h, 82% yield). Deprotection of the ribose was achieved by the action of tetrabutylammonium fluoride in THF at 0°C[8], leading to the expected pyrrole derivative **3** which was purified by chromatography on silica gel. Recrystallization in ethanol left a white powder (47% yield): mp 140°C; analysis $C_{15}H_{17}N_5O_4$; $[\alpha]_D^{20}$ - 54° (c, 1, in ethanol); ms (70 eV): m/z 331 (M^+), 314, 301, 242, 228, 199 (base peak) [peaks of ribose fragmentation[9]], 119 (purinium ion), 80 (methylpyrrolium ion); ^{1}H nmr (δ/TMS, DMSO d_6): 2.09 (s, 3H, CH₃), 3.63 (m, 2H , 5'CH₂), 3.9-4.3 (m, 2H, 3'H and 4'H), 4.58 (m, 1H, 2'H), 5.99 (d, 1H, 1'H), 6.23 (m, 1H, 4H pyrrole), 8.00 (m, 1H, 2H pyrr.), 8.12 (m, 1H, 5H pyrr.), 8.65 and 8.75 (2s, 2H,

2 and 8H purine); λ_{max} (EtOH 95%)(HCl 0.1 M) 300 nm (ε_{max} 23100), 293 (23300), 220 (sh) (13000), λ_{min} 247 (3700); λ_{max} (neutral) 300 (23700), 293 (24000), 220 (sh) (13700), λ_{min} 248 (4300); λ_{max} (NaOH 10^{-3} M) 300 (24000), 293 (24200), 220 (sh) (13200), λ_{min} 246 (4500).

The aglycone **6** of the pyrrolylpurine riboside **3** was synthesized through a similar route, from N-9 protected 6-chloropurine[10] **7** (scheme 2).

Scheme 2

The cyclized product **9** was easily deprotected by stirring in a 0.1 M HCl methanolic solution. Pure 6-(3-methylpyrrol-1-yl)purine **6** was obtained by recrystallization in ethanol (55% yield): mp 260°C; Analysis $C_{10}H_9N_5$; ms (70 eV): m/z 199 (M$^+$), 198 (M-H),[11] 171 (M-H-HCN), 159 (M-CH=C-CH$_3$), 119 (purinium ion), 92 (purinium - HCN), 80 (3-methylpyrrolium ion); nmr (δ/TMS, DMSO d$_6$) 2.10 (s, 3H, CH$_3$), 6.22 (m, 1H, 4H pyrrole), 8.07 (m, 1H, 2H pyrr.), 8.17 (m, 1H, 5H pyrr.), 8.52 and 8.62 (2s, 2H, 2 and 8H purine); λ_{max} (EtOH 95%): (HCl 0.1 M) 300 nm (ε_{max} 25100), 292 (24900), 260 (sh) (5200) ; (neutral) 299 (24500), 291 (24600), 256 (sh) (4600); (NaOH 10^{-3} M) 310 (sh) (17400), 301 (22300), 294 (sh) (19500), 221 (sh) (16700).

High resolution nmr spectra of compound **9** allowed us, through double irradiation experiments, to ascertain the 3-methylpyrrole structure of the purine 6-substituent found in the compounds **3, 6** and **9**.[$J_{2,5}$ = 1.6 Hz, J_{2,CH_3} = 1Hz, $J_{4,5}$ = 3.05 Hz; Litt. respectively 1.546, 0.929 and 2.593 Hz in 3-methylpyrrole[12]].

Compounds **3** and **6** were also obtained by direct oxidation of zeatin riboside and zeatin, using pyridinium dichromate in EtOAc-AcOH solution.[13] However, yields were low (16 and 10% respectively), due to incomplete reactions resulting from weak solubility of the starting compounds and to difficulties at the purification stage.

The isolated metabolite of zeatin riboside was identified with **3** by co-chromatography (tlc and hplc) and from its spectroscopic (uv and ms) properties, and its expected aglycone, obtained by acidic treatment, was identified with **6** by the same methods. Preliminary testing indicates that 6-(3-methylpyrrol-1-yl)-9-ß-D-ribofuranosylpurine has no cytokinin activity in a plant cell division assay and that it is, on the contrary, inhibitory of the growth of tobacco cell suspensions at concentrations above 2 µM.

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