SYNTHESIS AND ABSOLUTE CONFIGURATION OF THE GREEN ALGA CYTOKININ 2-HYDROXY-1'-METHYLZEATIN

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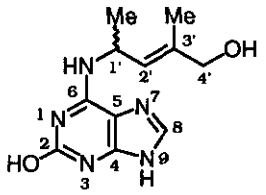
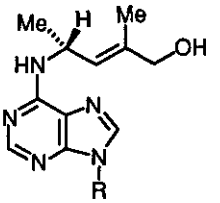
Abstract——The correctness of the gross structure of the marine green alga cytokinin 2-hydroxy-1'-methylzeatin (**1**) has been confirmed as a result of the chiral syntheses of (1'*R*)-**1** and (1'*S*)-**1**. An indirect comparison of the cytokinin activity of the natural cytokinin with those of the synthetic enantiomers suggests that the *R* configuration may be assigned to the natural one unless it would be racemic.

In 1990, Farooqi *et al.*[1] reported the isolation of a novel cytokinin from methanolic extracts of a marine green alga (code No. NIO-143) and proposed the gross structure **1** for it on the basis of spectroscopic data. However, they left the absolute stereochemistry at the asymmetric center in the side chain undetermined. This reminds us of a similar situation encountered recently in the structure determination of 1'-methylzeatin (**2**) and its 9-β-D-ribofuranosyl derivative (**3**), structurally analogous cytokinins from *Pseudomonas syringae* pv *savastanoi*,[2,3] whose absolute configurations were eventually established by means of chemical synthesis.[4] In this communication, we wish to record the results of our synthetic work, which have confirmed

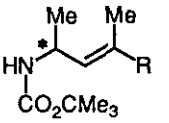
the correctness of the proposed gross structure and have led to a proposal for its absolute stereochemistry.



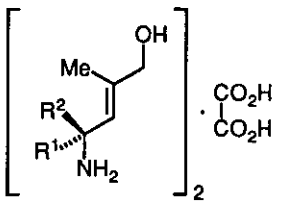
1



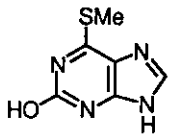
2: R = H
3: R = β-D-ribofuranosyl



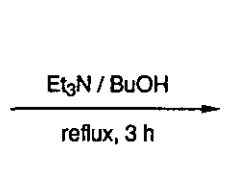
(*R*)- and (*S*)-**4**: R = CO_2Me
(*R*)- and (*S*)-**5**: R = CO_2H
(*R*)- and (*S*)-**6**: R = CH_2OH

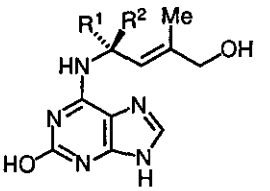


(*R*)-**7**: R^1 = Me, R^2 = H
(*S*)-**7**: R^1 = H, R^2 = Me



8





(*R*)-**1**: R^1 = Me, R^2 = H
(*S*)-**1**: R^1 = H, R^2 = Me

The key intermediates selected for the syntheses of both enantiomers of 2-hydroxy-1'-methylzeatin (**1**) were the chiral amine salts [(*R*)-**7** and (*S*)-**7**], and they were prepared from D- and L-alanines *via* the previously reported synthetic route,[4,5] proceeding through the α,β-unsaturated esters [(*R*)-**4** and (*S*)-**4**] and the allyl alcohols [(*R*)-**6** and (*S*)-**6**],[4] with some modification. In both chiral series, the conversion of **4** into **6** had previously been effected in two steps consisting of alkaline hydrolysis of **4** and $NaBH_4$ reduction of the resulting carboxylic acid (**5**) by the mixed anhydride method.[4] In the present study, however, reduction of (*R*)-**4** with diisobutylaluminum hydride (DIBAH) in CH_2Cl_2–hexane at –78°C for 75 min was found to give (*R*)-**6** [$[\alpha]_{365}^{23}$ –8.0° (*c* 1.00, MeOH)][6] in one step in 96% yield. This result is parallel to that obtained with the corresponding cis isomer.[5] A similar DIBAH reduction of (*S*)-**4** for 45 min afforded (*S*)-**6** [$[\alpha]_{365}^{25}$ +8.2° (*c* 1.01, MeOH)][7] in 96% yield.

Condensation of (*R*)-**7** with 2-hydroxy-6-methylthiopurine monohydrate (**8**•H_2O)[8] in boiling 1-butanol containing Et_3N for 3 h furnished (1'*R*)-2-hydroxy-1'-methylzeatin [(*R*)-**1**] [mp >300°C

(darkened at 275°C); $[\alpha]_D^{19}$ +41.6° (*c* 0.288, MeOH); cd (*c* 8.82×10^{-5} M, MeOH) $[\theta]^{19}$ (nm): –5900 (278) (neg. max.), +16300 (248) (pos. max.), +11400 (236) (neg. max.), +19000 (226) (pos. max.)][9] in 90% yield. A similar condensation of (*S*)-**7** with **8**•H_2O produced (1'*S*)-2-hydroxy-1'-methylzeatin [(*S*)-**1**] [mp >300°C (darkened at 275°C); $[\alpha]_D^{17}$ –38.1° (*c* 0.267, MeOH); cd (*c* 8.15×10^{-5} M, MeOH) $[\theta]^{19}$ (nm): +6260 (278) (pos. max.), –16000 (248) (neg. max.), –10600 (236) (pos. max.), –17700 (226) (neg. max.)][9] in 84% yield. The uv, ^{1}H nmr, and mass spectra of synthetic (*R*)-**1** or (*S*)-**1** were found to be virtually identical with those of the natural cytokinin (**1**), establishing that the latter is 2-hydroxy-1'-methylzeatin indeed. On the other hand, we were unable to establish the chiroptical identity on account of paucity of the natural cytokinin, thus leaving its absolute stereochemistry incomplete.

In a preliminary test for cytokinin activity using the tobacco callus bioassay,[4b] (*R*)-**1** was active at 1 μM concentration, whereas (*S*)-**1** was completely inactive at 0.01–10 μM concentration. Since the natural cytokinin at the crude extract level has also been shown to be active in the cucumber cotyledon greening bioassay,[1] it seems likely that the formula (*R*)-**1** is a complete expression for the green alga cytokinin unless it would be racemic. Interestingly, a plant growth factor produced by the fungus *Alternaria brassicae* has recently been assigned the gross structure **1**.[10]

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6. Lit.[4b] $[\alpha]_{365}^{16}$ –8.4° (*c* 1.00, MeOH).
7. Lit.[4b] $[\alpha]_{365}^{17}$ +7.9° (*c* 1.00, MeOH).
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