1'-METHYL-*trans*-ZEATIN AND ITS ANALOGUES: THEIR OCCURRENCE, CHEMISTRY AND SYNTHESIS, AND CYTOKININ ACTIVITY†

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Abstract — Recent advances in isolation, structure establishment, chemistry and synthesis, and evaluation of cytokinin activity of the title compounds are reviewed with 45 reference citations.

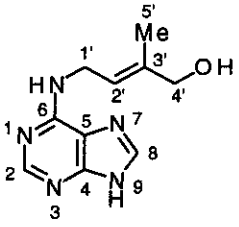
CONTENTS

I. Introduction

Cytokinins are a group of phytohormones characterized primarily by the ability to pro-

†This review article is dedicated to the memory of Emeritus Professor Dr. Shun-ichi Yamada (University of Tokyo), who passed away at the age of 80 on April 21, 1996.

mote cell division in plant tissue cultures or secondarily by the ability to promote seed germination, leaf and cotyledon growth, or lateral bud development, to inhibit chlorophyll degradation, or to induce buds on moss protonema.[1] Besides a large number of synthetic cytokinins, whose activity varies from highly active to almost inactive, more than 30 naturally occurring cytokinins have so far been isolated from plants and microorganisms, and their chemical structures established.[1c,2–10] Interestingly, all these *natural cytokinins are* N^6*-substituted adenines* with or without substituent(s) on the purine nucleus.[11] They may be structurally classified into six families according to their N^6-substituents: (1) the *trans*-zeatin (which is often referred to simply as zeatin) family (*e. g.*, type **1**); (2) the *cis*-zeatin family (*e. g.*, type **2**); (3) the dihydrozeatin family (*e. g.*, type **3**); (4) the IPA [N^6-(Δ^2-isopentenyl)adenine; N^6-(3-methyl-2-butenyl)adenine; N^6-(γ,γ-dimethylallyl)adenine] family (*e. g.*, type **4**); (5) the N^6-(2-hydroxybenzyl)adenine family (*e. g.*, type **5**); (6) the 1'-methyl-*trans*-zeatin family [*e. g.*, type (1'*R*)-**6**].[12]



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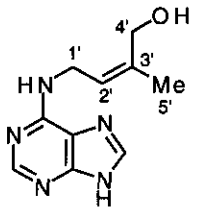

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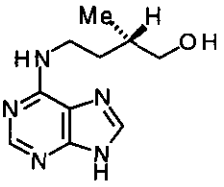

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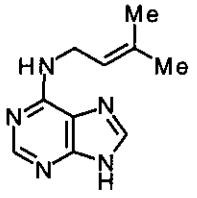

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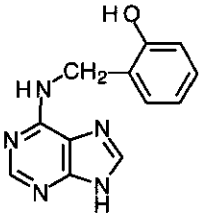

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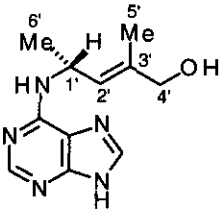


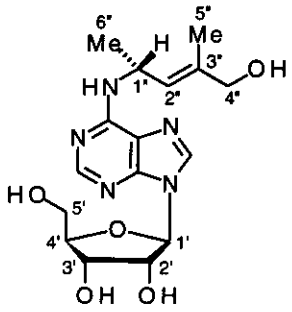
(1'*R*)-**6**

The last family in the above classification is a relative newcomer into the natural cytokinin group, and its members are characterized by their N^6-substituents consisting of a branched allylic alcoholic C_6-unit with an asymmetric center adjacent to the N^6 atom. The aim of this article is to give an overview of this unique newcomer and its analogues so far synthesized, with coverage of the literature through the late part of 1996.

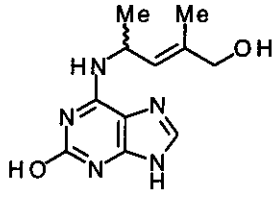
II. Occurrence

Pseudomonas syringae pv *savastanoi*, the causative organism of olive, oleander, privet, and ash knot, secretes several cytokinins.[2,13–16] In 1985, Surico *et al.*[2] reported the isolation of two new cytokinins and two other known cytokinins, *trans*-zeatin (**1**) and its 9-β-D-ribofuranoside, from AcOEt extracts of the culture filtrate of this gall-forming

phytopathogenic bacterium. They proposed the gross structures (**6**) (1'-methyl-*trans*-zeatin)[3] and (**7**) (1"-methyl-*trans*-zeatin 9-β-D-ribofuranoside),[2] without specifying the absolute configurations at the 1'- and 1"-positions, respectively, for the new cytokinins on the basis of spectroscopic data and comparison with related adenine derivatives including **1** and its 9-β-D-ribofuranosyl derivative. The accumulation of **6** and **7** in the culture medium of *P. syringae* pv *savastanoi* has been investigated.[14,16] In addition, naturally occurring cytokinins (including the above four) produced by two phytopathogenic *Pseudomonas* bacteria and some of their derivatives and analogues have been characterized at the submicrogram level by FAB-MS/MS.[17] Evidente *et al.*[18] further found **7**, *trans*-zeatin 9-β-D-ribofuranoside, **3**, and its 9-β-D-ribofuranoside in the culture of an atypical ash strain of *Pseudomonas syringae* subsp. *savastanoi*.



(1"*R*)-**7**



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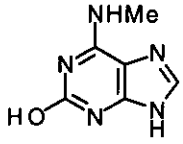

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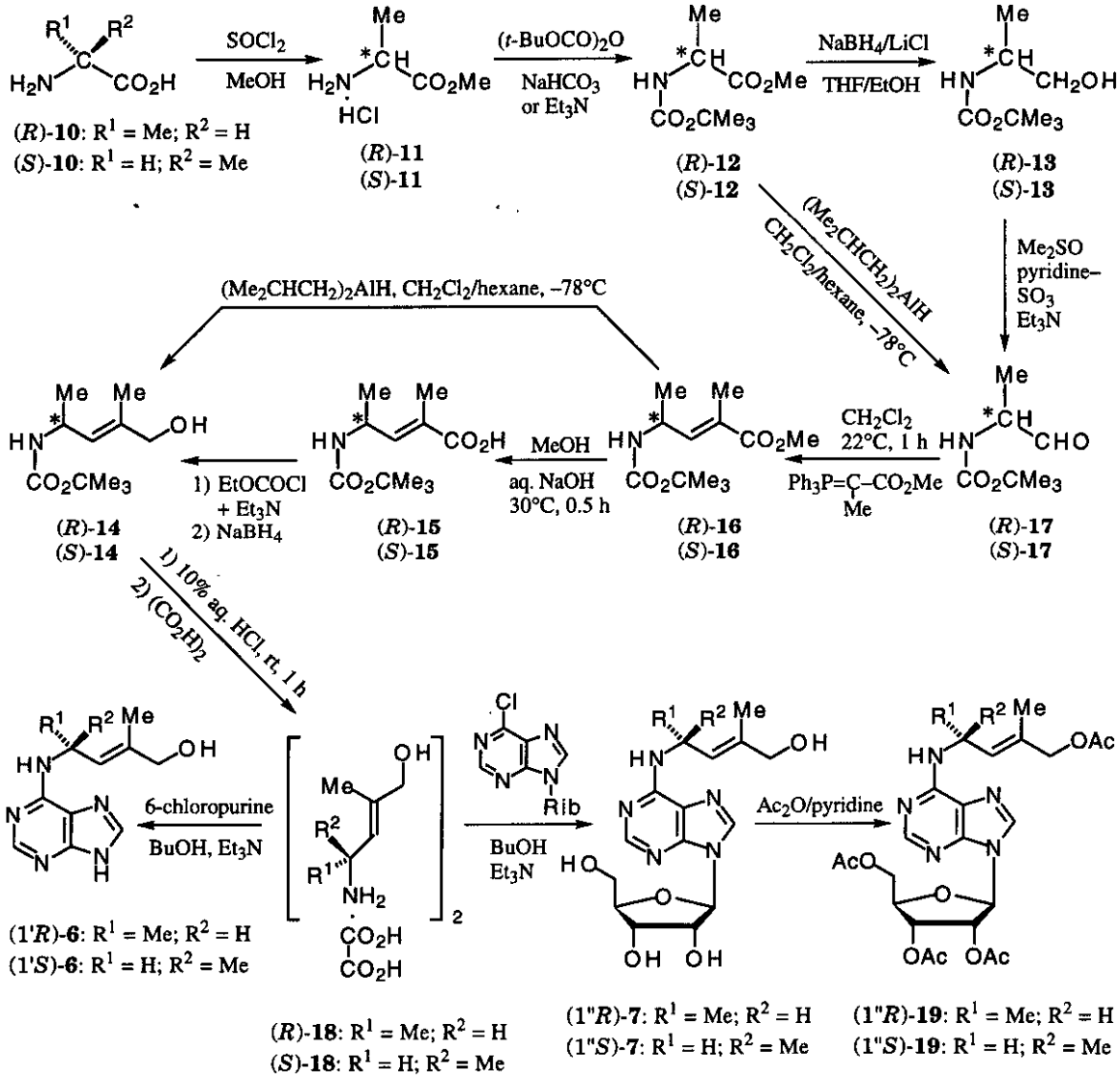
In 1990, Farooqi *et al.*[6a] reported the isolation of a new cytokinin from methanolic extracts of a marine green alga (code No. NIO-143) and another new cytokinin from blue coral (NIO-156). They proposed the gross structure (**8**) (2-hydroxy-1'-methyl-*trans*-zeatin: absolute configuration unknown) for the former and the structure (**9**) (2-hydroxy-N^6-methyladenine) for the latter on the basis of spectroscopic data.[6a] Furthermore, a plant growth factor produced by the fungus *Alternaria brassicae* has recently been assigned the gross structure (**8**) by Dahiya and Tewari.[7]

More recently, Farooqi *et al.*[19] identified cytokinin-like substances in methanolic extracts of the leaves (at the vegetative bud stage) of *Rosa damascena* Mill. as **9**, N^6-methyldihydrozeatin 9-β-D-ribofuranoside, **1**, and **4**.

III. Chemistry and Synthesis

A. Chiral and Racemic 1'-Methyl-*trans*-zeatins

The structure and stereochemistry of the natural cytokinin (–)-1'-methyl-*trans*-zeatin have been established as (1'*R*)-**6**, as a result of the chiral syntheses of (1'*R*)-**6** and (1'*S*)-**6** by Fujii's group.[4a,b] The key intermediates selected for the syntheses of the candidate structures were the chiral amine salts [(*R*)-**18** and (*S*)-**18**], and they were prepared by



Scheme 1

incorporating the skeletons and chirality of D- and L-alanines [(*R*)-**10** and (*S*)-**10**] into them, as shown in Scheme 1.[4a,b,6b,c,20] Since the enantiomeric purity of the sample of (*R*)-**16** thus obtained was determined to be more than 96%, those of (*R*)-**18** and (*S*)-**18** should be equally high, provided that each of conversions beyond **16** involved no racemization. Separate purinylations of (*R*)-**18** and (*S*)-**18** with 6-chloropurine in boiling 1-butanol containing Et_3N for 10 h gave (1'*R*)-**6** [mp 201–202°C; $[\alpha]_D^{26}$–109° (*c* 0.153, EtOH)] and (1'*S*)-**6** [mp 201–202°C; $[\alpha]_D^{26}$+103° (*c* 0.137, EtOH)] in 70% yield each. Of the two optical isomers, (1'*R*)-**6** was identical with natural (–)-1'-methyl-*trans*-zeatin by comparison of their chiroptical properties, MS, UV, and ^1H and ^{13}C NMR spectra, and TLC mobilities.

A parallel sequence of reactions starting from (±)-alanine [(±)-**10**] and proceeding through (±)-**11**, (±)-**12**, (±)-**13**, (±)-**17**,[21] (±)-**16**, (±)-**15**, (±)-**14**,[6c] and (±)-**18** furnished racemic 1'-methyl-*trans*-zeatin [(±)-**6**] (mp 175.5–176.5°C).[4c]
Some reactions of (1'*R*)-**6** will be described in Section IV,D.

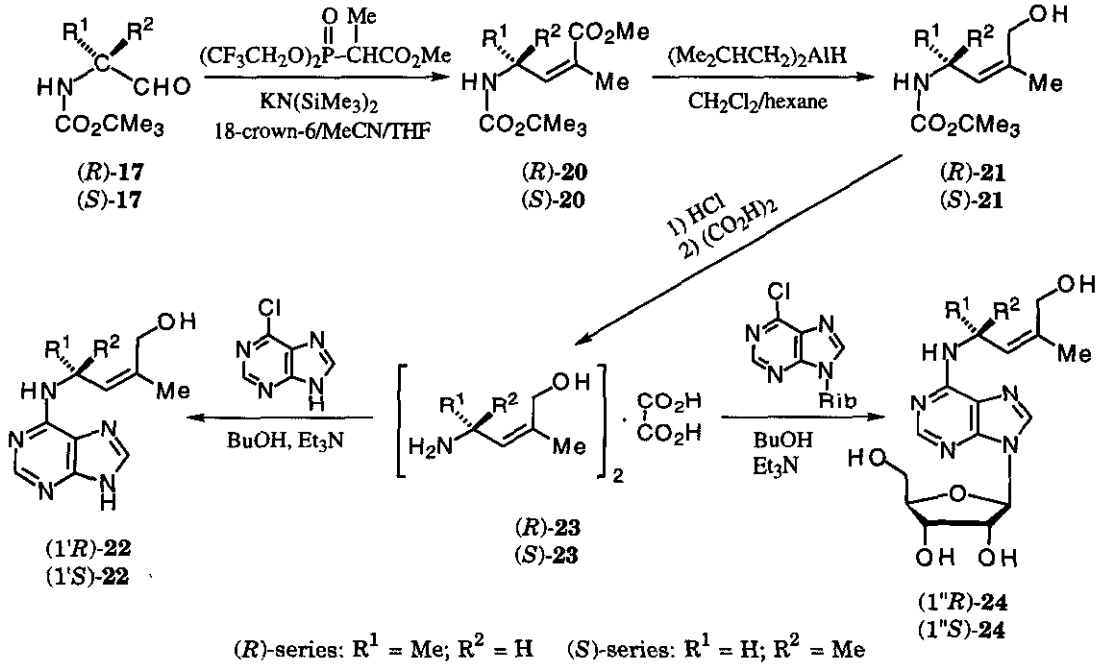
B. THE 9-β-D-RIBOFURANOSIDES OF CHIRAL 1'-METHYL-*trans*-ZEATINS

The Italian research group has reported that hydrolysis of natural **7** with 0.5 M HCl at 95°C for 60 min or 6 h yielded β-D-ribose or the aglycone (**6**), respectively, as checked by TLC analysis.[2,3] The solution of the structure and absolute stereochemistry of natural **7** came with the syntheses of (1"*R*)-**7** and (1"*S*)-**7** by Fujii's group, who eventually found that the former structure [(1"*R*)-**7**] is a complete expression for the natural glycosidic cytokinin.[4a,b] As delineated in Scheme 1, separate condensations of 6-chloro-9-β-D-ribofuranosylpurine with (*R*)-**18** and with (*S*)-**18** were effected in boiling 1-butanol containing Et_3N for 8 h, giving (1"*R*)-**7** [hemihydrate mp 130–132°C; $[\alpha]_D^{14}$–117° (*c* 0.102, MeOH)] and (1"*S*)-**7** [$[\alpha]_D^{18}$–2.2° (*c* 0.50, MeOH)] in 88% and 89% yields, respectively. The two nucleosides were then acetylated with acetic anhydride and pyridine at 30°C for 2 h to provide the tetra-*O*-acetyl derivatives [(1"*R*)-**19** and (1"*S*)-**19**] in 95% and 80% yields, respectively. The synthetic (1"*R*)-**7** and (1"*R*)-**19** were found to be identical (by comparison of the spectra, TLC mobility, and chiroptical property) with natural **7** and its tetra-*O*-acetyl derivative, respectively.
Previous carbon-13 chemical shift assignments[2,3] for the two methyl carbons in the N^6-substituent of (1'*R*)-**6** and (1"*R*)-**7**, as well as those[2] for the C(2') and C(3') of (1"*R*)-**7**, have been reversed by comparison with the ^{13}C NMR data for the diastereomeric nucleoside [(1"*S*)-**7**] and the *cis* isomers [(1'*R*)-**22** (Section III,C) and (1"*R*)-**24** (Section III,D)].[4d]
Some other reactions of (1"*R*)-**7** will be described in Section IV,D.

C. CHIRAL 1'-METHYL-*cis*-ZEATINS

The natural occurrence of both the *cis* and *trans* isomers (**2** and **1**) in the 1'-unsubstituted zeatin series suggests that the *cis* isomers of (1'*R*)-**6** and (1"*R*)-**7** may also occur in nature, and the availability of synthetic reference samples would greatly facilitate the search for these *cis* isomers as natural products. Thus, Fujii's group synthesized both (1'*R*)-1'-methyl-*cis*-zeatin [(1'*R*)-**22**][20] and its enantiomer [(1'*S*)-**22**][6c] according to a route shown in Scheme 2.
For the synthesis of (1'*R*)-**22**,[20] the conversion of (*R*)-**17** into the (*Z*)-ester [(*R*)-**20**] with excellent *Z* stereoselectivity was a key process in the synthetic scheme, and this was feasible by applying the Still–Gennari modification[22] [$(CF_3CH_2O)_2P(O)CH(Me)CO_2Me$, $KN(SiMe_3)_2$, 18-crown-6/MeCN, THF, –78°C, 30 min] of the Horner–Wadsworth–Emmons reaction. On reduction with DIBALH in CH_2Cl_2–hexane at –78°C for 45 min, (*R*)-**20** gave the (–)-allylic alcohol [(*R*)-**21**] in 92% yield. Acid hydrolysis of (*R*)-**21** (10% aqueous HCl, rt, 1 h) and isolation of the product in the form of the oxalate furnished (*R*)-**23** in 88% yield. Finally, purinylation of (*R*)-**23** with 6-chloropurine in boiling 1-butanol

containing Et_3N for 3.5 h afforded (1'*R*)-**22** [mp 182–183.5°C; $[\alpha]_D^{22}$–128° (*c* 0.117, EtOH)] in 75% yield. Repetition of this synthetic route, but starting with (*S*)-**17** instead of (*R*)-**17**, gave (1'*S*)-**22** [mp 179.5–181°C; $[\alpha]_D^{20}$+131° (*c* 0.148, EtOH)] in good overall yield.[6c]



Scheme 2

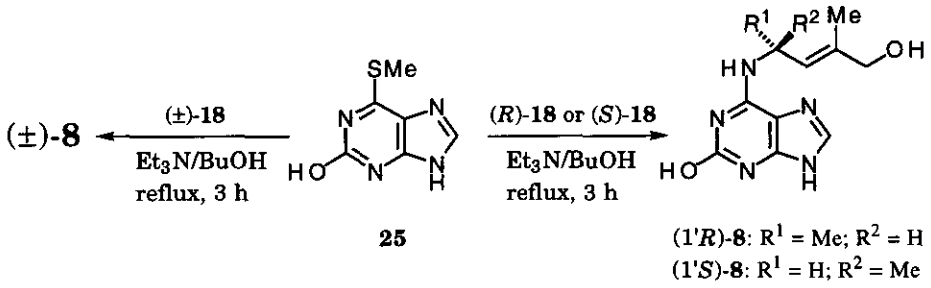
D. The 9-β-D-Ribofuranosides of Chiral 1'-Methyl-*cis*-zeatins

For the same reason as that described in Section III,C, Fujii's group prepared (1"*R*)-1"-methyl-*cis*-zeatin 9-β-D-ribofuranoside [(1"*R*)-**24**][20] and (1"*S*)-1"-methyl-*cis*-zeatin 9-β-D-ribofuranoside [(1"*S*)-**24**].[6c] As shown in Scheme 2, separate condensations of 6-chloro-9-β-D-ribofuranosylpurine with (*R*)-**23** and with (*S*)-**23** in boiling 1-butanol containing Et_3N for 3.5 h gave (1"*R*)-**24** [mp 206.5–207.5°C; $[\alpha]_D^{23}$–131° (*c* 0.156, MeOH)] and (1"*S*)-**24** [mp 134–136.5°C; $[\alpha]_D^{24.5}$+13.1° (c 0.161, MeOH)] in 96% and 83% yields, respectively.

E. Chiral and Racemic 2-Hydroxy-1'-methyl-*trans*-zeatins

The problem of the structure of natural 2-hydroxy-1'-methyl-*trans*-zeatin (**8**)[6a] has been essentially the same as those of natural **6** and **7**. Fujii *et al.*[6b,c] synthesized (1'*R*)-**8**, (1'*S*)-**8**, and (±)-**8** from 2-hydroxy-6-methylthiopurine (**25**) through a route shown in Scheme 3.

The MS, UV, and ^1H NMR spectra of the synthetic (1'*R*)-**8**, (1'*S*)-**8**, or (±)-**8** were found to be virtually identical with those of the marine green alga cytokinin (**8**),[6a] establishing that the natural cytokinin is indeed 2-hydroxy-1'-methyl-*trans*-zeatin. However, they were unable to establish the chiroptical identity on account of paucity of the natural



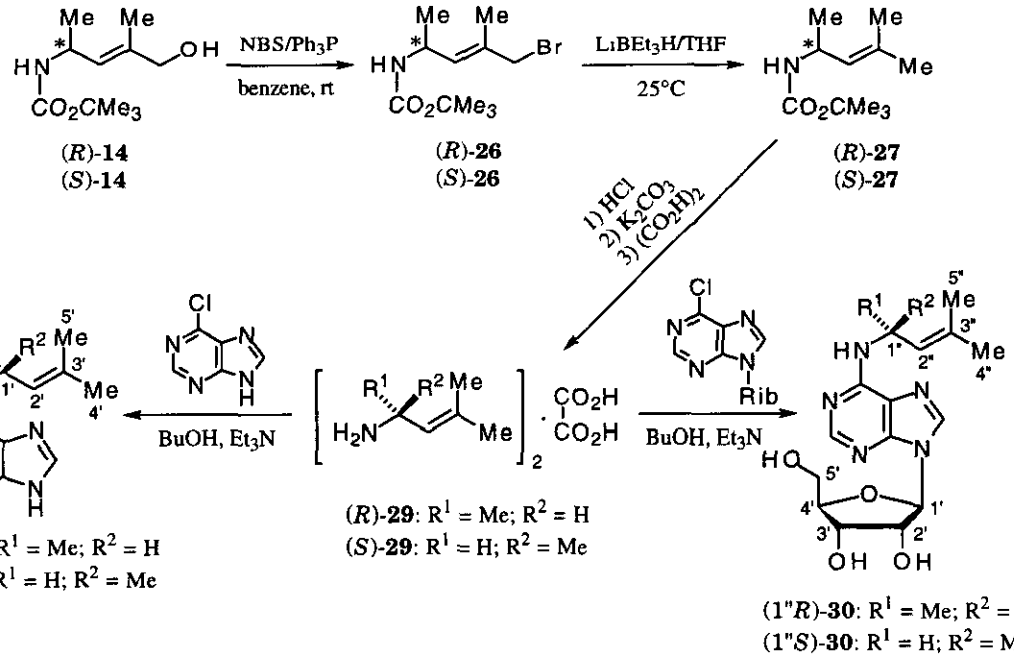
Scheme 3

cytokinin, thus leaving its absolute stereochemistry unknown. As will be described in Section V, however, in the tobacco callus bioassay the above synthetic (1'*R*)-**8** and (±)-**8** were active at 1 μM and 4 μM concentrations, respectively, whereas the synthetic (1'*S*)-**8** was completely inactive at 0.01–10 μM concentration.[6b,c] In the lettuce seed germination bioassay (1'*R*)-**8** was very weakly active, whereas (1'*S*)-**8** was completely inactive.[23] Since the green alga cytokinin at the crude extract level has been shown to be active in the cucumber cotyledon greening bioassay,[6a] it seems likely that the formula [(1'*R*)-**8**] is a complete expression for this natural cytokinin unless it is racemic.[6b,c]

IV. Related Compounds

A. CHIRAL N^6-(1,3-DIMETHYL-2-BUTENYL)ADENINES

The natural occurrence of the 1'-methylated *trans*-zeatin family suggests that analo-



Scheme 4

gously methylated derivatives in the IPA family (Section I) may also exist in nature. If synthetic reference samples were available, the search for such 1'-methyl or 1"-methyl analogues as natural products would be greatly facilitated. For this reason, together with their continuing interest in the preparation and structure–activity relationships of cytokinins,[24] Fujii's group synthesized both enantiomers of N^6-(1,3-dimethyl-2-butenyl)adenine (1'-methyl-IPA) (**28**), as shown in Scheme 4.[21]

Thus, treatment of (*R*)-**14** with NBS in benzene in the presence of triphenylphosphine at rt for 50 min afforded the allylic bromide [(*R*)-**26**] in 83% yield. Reduction of (*R*)-**26** was effected with Super-Hydride in THF at 25°C for 30 min, giving (*R*)-**27** in 80% yield. The carbamate [(*R*)-**27**] was then hydrolyzed with aqueous HCl in EtOH at rt for 7 h, and the basic product was isolated in the form of the oxalate [(*R*)-**29**] in 59% yield. Purinylation of (*R*)-**29** with 6-chloropurine in boiling 1-butanol containing Et_3N for 3 h furnished (1'*R*)-1'-methyl-IPA [(1'*R*)-**28**] in 92% yield.

A parallel sequence of reactions starting from (*S*)-**14** provided (*S*)-**26** (73% yield), (*S*)-**27** (80%), (*S*)-**29** (56%), and (1'*S*)-1'-methyl-IPA [(1'*S*)-**28**] (86%).

B. The 9-β-D-Ribofuranosides of Chiral N^6-(1,3-Dimethyl-2-butenyl)adenines

Fujii's group also prepared the 9-β-D-ribofuranosides of (1'*R*)-**28** and (1'*S*)-**28** for the same reason as that described in Section IV,A. Thus, separate condensations of 6-chloro-9-β-D-ribofuranosylpurine with (*R*)-**29** and with (*S*)-**29** in boiling 1-butanol containing Et_3N for 5–7 h gave the target nucleosides [(1"*R*)-**30** and (1"*S*)-**30**] in 98% and 74% yields, respectively (Scheme 4).[21]

C. 2-Hydroxy-N^6-methyladenine

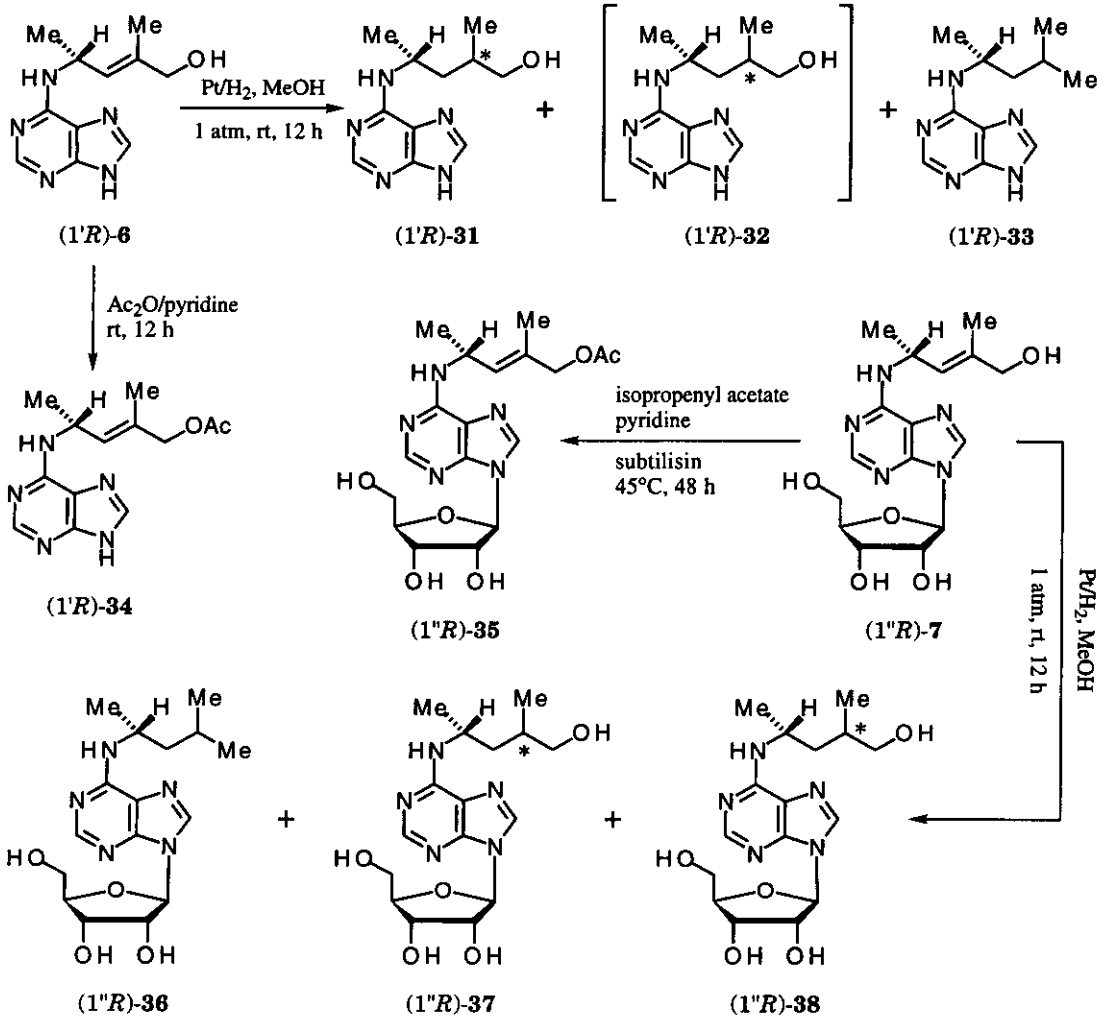
The candidate structure (**9**) for the new cytokinin from blue coral[6a] has been synthetically known since 1968.[25] Fujii's group prepared **9** from 2-hydroxy-6-methylthiopurine (**25**) in 85% yield by treating the latter with boiling aqueous methylamine for 1 h, a procedure slightly modified from that[25] reported. The synthetic **9** was found to be identical (by comparison of the MS, UV, and ^1H NMR spectra) with a sample of the natural cytokinin.

D. Other Compounds

The total syntheses of (1'*R*)-1'-methyl-*trans*-zeatin [(1'*R*)-**6**] and its 9-β-D-ribofuranoside [(1"*R*)-**7**], described in Section III,A,B, have made it possible to secure sufficient amounts of these two natural cytokinins for the study of structure–activity relationships. Thus, Evidente *et al.*[26] prepared some derivatives of (1'*R*)-**6** and (1"*R*)-**7** in the following manner (Scheme 5).

Catalytic hydrogenation of (1'*R*)-**6** gave (1'*R*)-2',3'-dihydro-1'-methylzeatin [(1'*R*)-**31**] and (1'*R*)-2',3'-dihydro-1'-methyl-IPA [(1'*R*)-**33**]. The absolute configuration of (1'*R*)-**31** at the 3'-position was inferred to be *S*. The probable (3'*R*)-diastereomer [(1'*R*)-**32**] could not be isolated from the hydrogenation mixture, although its formation was suggested by TLC

analysis. Treatment of (1'*R*)-**6** with acetic anhydride and pyridine afforded (1'*R*)-4'-*O*-acetyl-1'-methyl-*trans*-zeatin [(1'*R*)-**34**].



Scheme 5

Next the nucleoside [(1"*R*)-**7**] was enzymatically converted into the 4"-*O*-acetyl derivative [(1"*R*)-**35**] by treatment with isopropenyl acetate in pyridine in the presence of subtilisin at 45°C for 48 h. Catalytic hydrogenation of (1"*R*)-**7** produced (1"*R*)-2",3"-dihydro-1"-methyl-IPA 9-riboside [(1"*R*)-**36**] and two diastereomeric (1"*R*)-2",3"-dihydro-1"-methylzeatin 9-ribosides [(1"*R*)-**37** and (1"*R*)-**38**].

V. Cytokinin Activity

The synthetic 1'-methylzeatin and its analogues described above were tested for cytokinin activity in the tobacco callus bioassay, lettuce seed germination bioassay, and/or etiolated cucumber cotyledon bioassay.

In the tobacco callus bioassay, the "natural" aglycone [(1'*R*)-**6**] was the most active in the 1'-methyl-*trans*-zeatin group (Section III,A,B):[4b] it was as active as the known 1'-unsubstituted cytokinin *trans*-zeatin (**1**). Interestingly, the "unnatural" aglycone [(1'*S*)-**6**] was apparently less active than (1'*R*)-**6**: the maximal yield of the callus was obtained at 0.04 μM (1'*R*)-**6** and at 1 μM (1'*S*)-**6**. This stereochemistry–activity relationship also holds at the nucleoside (**7**) and the tetra-*O*-acetyl derivative (**19**) levels. As expected,[1b,d] the nucleoside [(1"*R*)-**7**] and its tetra-*O*-acetyl derivative [(1"*R*)-**19**] were all less active than the aglycone [(1'*R*)-**6**]. However, the corresponding triplets [(1"*S*)-**7**, (1"*S*)-**19**, and (1'*S*)-**6**] in the (*S*)-series had similar activities. Thus, the cytokinin activity in the 1'-methyl-*trans*-zeatin group follows the order: *trans*-zeatin (**1**) ≈ (1'*R*)-**6** > (1"*R*)-**7** ≈ (1"*R*)-**19** > (1'*S*)-**6** ≈ (1"*S*)-**7** ≈ (1"*S*)-**19**.[4b] The racemic aglycone [(±)-**6**] was active at 0.04–1 μM concentration, a range between the optimum concentrations of both enantiomers [(1'*R*)-**6** and (1'*S*)-**6**].[4c] In the 1'-methyl-*cis*-zeatin group (Section III,C,D) and the 2-hydroxy-1'-methyl-*trans*-zeatin group (Section III,E), the maximal yield of the callus was obtained at 1 μM (1'*R*)-**8**; 1–4 μM (±)-**8**; 4 μM (1'*R*)-**22**; 40 μM (1'*S*)-**22**; 100 (or >100) μM (1"*R*)-**24**.[6c,20b] Both (1'*S*)-**8** and (1"*S*)-**24** were completely inactive at 0.01–10 μM and 0.1–100 μM concentrations, respectively.[6c] 2-Hydroxy-N^6-methyladenine (**9**) (Section IV,C) was very weakly active at 100 μM concentration.[6c] In the 1'-methyl-IPA group (Section IV,A,B), the maximal yield of the callus was obtained at 0.04–0.1 μM IPA (**4**); 0.4–1 μM (1'*R*)-**28**; 1 μM (1"*R*)-**30**; 1 μM IPA 9-riboside; 1–4 μM (1'*S*)-**28**; 4 μM (1"*S*)-**30**; 4–10 μM *cis*-zeatin 9-riboside.[21]

Interestingly, in all cases the *R* configuration at the 1'- or 1"-position seems to be more important than the *S* configuration in determining cytokinin activity. It is also apparent that introduction of a hydroxy group into 1'-methyl-*trans*-zeatin at the 2-position in both the (1'*R*)- and (1'*S*)-series reduces the activity to a considerable extent.

In the lettuce seed germination bioassay, a similar activity order was found for the above compounds.[4b,20b,23]

In the etiolated cucumber cotyledon bioassay carried out by Evidente *et al.*,[26] (1'*R*)-**6** and (1"*R*)-**7** displayed a higher stimulating potency of chlorophyll synthesis when compared to *trans*-zeatin (**1**) and its 9-β-D-riboside, respectively. With regard to the synthetic analogues of (1'*R*)-**6** and (1"*R*)-**7**, any modification of the carbon skeleton of the N^6-substituent proved to influence the activity. In particular, (1'*R*)-**31**, (1"*R*)-**37**, and (1"*R*)-**38** obtained by saturation of the double bond in (1'*R*)-**6** and (1"*R*)-**7** showed a reduction or the complete loss of activity. (1'*R*)-2',3'-Dihydro-1'-methyl-IPA [(1'*R*)-**33**] and its 9-riboside [(1"*R*)-**36**] were inactive. The acetylation of the hydroxy group as in the cases of (1'*R*)-**34** and (1"*R*)-**35** did not significantly affect the biological activity.

VI. Conclusions

The occurrence, chemistry and synthesis, and cytokinin activity of 1'-methyl-*trans*-zeatin and its analogues highlighted in this review appear to have occupied a unique

niche in the cytokinin chemistry during the last 10 years. The above results provide a fine example in support of the statement[27] that chemical synthesis can still be an important and powerful tool for structure elucidation of natural products (particularly of those isolated only in minute quantity, unstable, and hard to crystallize) even in this new era of highly refined spectroscopic studies.

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