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CYTOKININS WITH DIFFERENT CONNECTING LINKS BETWEEN PURINE AND ISOPENTENYL OR BENZYL GROUPS

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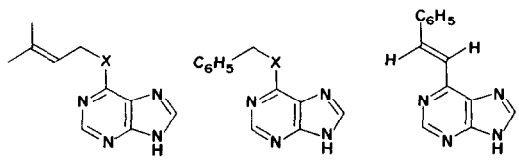
Key Word Index—Biological activity; cytokinins; purine, 6-(4-methyl-3-pentenyl), 6-(2-phenethyl), 6-*trans*-styryl; purines, 6-benzyl-amino, -oxy, -thio; purines, 6-(3-methyl-2-butenyl-amino, -oxy, -thio); synthesis.

Abstract—Using the tobacco bioassay a comparison was made between the cytokinin activities of the following series of compounds with different connecting links (6-NH, S, O, CH_2) between the purine ring and isopentenyl or benzyl groups: 6-(3-methyl-2-butenylamino)purine (**1a**), 6-(3-methyl-2-butenylthio)purine (**1b**), 6-(3-methyl-2-butenyloxy)purine (**1c**), and 6-(4-methyl-3-pentenyl)purine (**1d**); 6-benzylaminopurine (**2a**), 6-benzylthiopurine (**2b**), 6-benzyloxypurine (**2c**), and 6-(2-phenethyl)purine (**2d**); also 6-*trans*-styrylpurine (**3**), the synthetic precursor of **2d**. All possess cytokinin activity, thus providing evidence that the intact base, consisting of nucleus and sidechain at the purine 6-position, is necessary and sufficient for such activity as measured in the tobacco bioassay. The biological activity in the 6-(3-methyl-2-butenyl-*X*)purine series decreases as a function of the linkage group in the order $X = NH > CH_2 > S \gg O$ and in the 6-benzyl-*X*-purine series in the order $X = NH \geq CH_2 = O \gg S$. The 6-*trans*-styrylpurine (**3**) is about equally active as 6-(2-phenethyl)purine (**2d**).

INTRODUCTION

For an understanding of the role of N^6-substituted adenines in exhibiting cytokinin activity [1,2], it is important to know whether expression of the activity involves the entire base (e.g. **1a**) or possible transfer of the sidechain from the nucleus at some stage [3]. By double labeling experiments, the synthetic cytokinin 6-benzylaminopurine (**2a**) has been shown to remain intact when incorporated at a very low level into the *t*RNA of growing tobacco callus tissue [4]. It has also been shown that the addition of a ribosyl moiety in the 9 position of exogenously supplied cytokinins is not a prerequisite for their promotion of cell division and growth of plant tissue [5]. Moreover, structure-activity studies centered on modification of both the sidechain and the nucleus have shown that activity-enhancing changes tend to be additive [6,7]. We therefore decided to synthesize a series of substituted purines designed to test the growing evidence that the intact base, consisting of nucleus and sidechain at the 6-position, is necessary and sufficient for cytokinin activity as measured in the tobacco bioassay.



(1) (2) (3)

X = a, NH; b, S; c, O; d, CH_2

We selected for comparative study a series of compounds (**1**) in which the connecting link between the Δ^2-isopentenyl group and the 6-substituted purine nucleus was NH, S, O and CH_2 and a similar series (**2**) in which the same linkages joined the benzyl group and the purine nucleus. It is highly unlikely that a common enzymatic pathway might effect cleavage of each of these sidechains, especially of the all-carbon sidechains in **1**d and **2**d. In the tobacco bioassay, 6-(3-methyl-2-butenylamino)purine (**1**a) and 6-benzylaminopurine (**2**a) gave detectable growth responses at about 1 nM, 6-(3-methyl-2-butenylthio)-purine (**1**b) at 100 nM (and is therefore about one-hundredth as active), while adenine alone required a concentration of >200 μM for a detectable response [8]. It remained to expand the series **1** and **2** by synthesis and to run a direct comparison of the cytokinin activities of all the compounds.

RESULTS AND DISCUSSION

Synthesis

Compounds **1**a and **2**a are available commercially and have been made many times in our laboratories. Compounds **1**b, **2**b, **1**c and **2**c have been described elsewhere. 6-*trans*-Styrylpurine (**3**), the precursor for compound **2**d, was prepared by the condensation of benzaldehyde with 6-methylpurine in ethanolic HCl according to the general method described for methylpyrimidine-type compounds [9], and applied to 6-methylpurine by Hampton [10]. The half of the AB pattern expected for the –CH=CH– group in compound **3** that was readily discernible in the PMR spectrum was a doublet with J 16 Hz, indicative of *trans* coupling. The long wavelength of the UV absorption also supported the *trans* structure (**3**). Catalytic hydrogenation of 6-*trans*-styrylpurine produced the target compound, 6-(2-phenethyl)-purine (**2**d), the structure of which was established by source, microanalysis, M^+ in the MS, NMR, and the similarity of its UV spectrum to that of 6-methylpurine [11]. 6-(4-Methyl-3-pentenyl)purine (**1**d) was made by the alkylation of the dianion of 6-methylpurine with 3-methyl-2-butenyl bromide under conditions employed by Murray *et al.* [12] with methylpyrimidine derivatives.

Cytokinin activity

A summary of the cytokinin activities found in all assays of the nine tested compounds is given in Fig. 1. A gradation of decreasing activity is apparent for the 6-(3-methyl-2-butenyl-*X*)purine series, with the naturally occurring compound 6-(3-methyl-2-butenylamino)purine (**1**a) being the most active. The methylene analog (**1**d) is about 20% as active as **1**a, the thio analog (**1**b) [8] about 4% as active, and the oxy analog (**1**c) only about 0·05% as active as **1**a. In fact the latter, **1**c, must be present in a concentration of 60 μM or greater for maximum yields of tissue. In the 6-benzyl-*X*-purine series, the three benzyl derivatives, containing the NH, O and CH_2 linkage groups, and 6-*trans*-styrylpurine (**2**a, **2**c, **2**d and **3**) have nearly equal activities, while the thio analog (**2**b) is only about 1% as active. Nevertheless, as indicated by the yield vs concentration curves from typical experiments, shown in Fig. 2, the latter compound (**2**b) when used in sufficiently high concentration (2–5 μM) gives about the same fr. wt yield as the four more active members of the series.

It is unlikely that these compounds would be such active cytokinins if their activity depended on cleavage of the sidechain from the purine nucleus. Nor is it likely that the tobacco callus

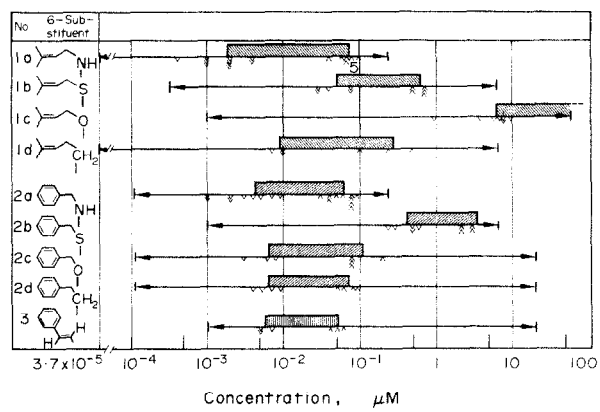


Fig. 1. Summary of the cytokinin activities of 6-(3-methyl-2-butenylamino)purine, its 6-thio, oxy, and methylene analogs, and of 6-benzylaminopurine, its 6-thio, oxy, and methylene analogs, as well as of 6-*trans*-styrylpurine in the tobacco assay. Compounds are identified by numbers as in the text, and the structures of the 6-substituents have also been indicated to facilitate comparison. The base lines represent the tested concentration range for each compound, and the bars represent the mean range over which growth increased as a nearly linear function of the log of concentration. Arrows represent the start and end points of this range for individual experiments.

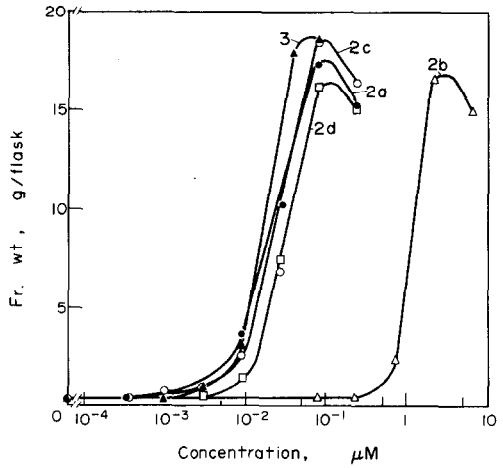


Fig. 2. Comparison of the cytokinin activities of 6-benzylaminopurine (**2a**) and its 6-thio (**2b**), oxy (**2c**) and methylene (**2d**) analogs and of 6-*trans*-styrylpurine (**3**). Compounds are identified by number as in the text and Fig. 1. Data are from Experiment C124, 8 February to 15 March 1973, except curve **3**, which is from Experiment C227, 31 May to 5 July 1973.

enzymes would cleave this assortment of natural and synthetic compounds with nearly equal facility. Indeed if the isopentenyl and benzyl moieties had been released from the thio derivatives the toxicity of the resulting 6-mercaptopurine would most probably have prevented the expression of any cytokinin activity by these compounds.

No explanation is provided for the relatively low cytokinin activities of compounds **1c** (with the *O* linkage group in the isopentenyl series) and **2b** (with the *S* linkage group in the benzyl series) as compared with the activities of the other seven compounds. Relatively low activities for **2b** and also for 6-furfurylthiopurine, as compared with kinetin, have been reported by Kuraishi [13] in radish leaf growth tests, while in tests with tobacco tissue both 6-benzylthiopurine and furfurylthiopurine were active, but apparently several-fold less active than kinetin [14].

The high potency and vigorous callus growth promoted by **1d** and **2d**, which contain the CH_2 linkage group, suggest that these compounds may be excellent synthetic cytokinins for practical use. As deaza compounds they have much greater activity than the 1-deaza and 3-deaza analogs of kinetin and 6-(3-methyl-2-butenylamino)purine [15]. As a family, compounds **1**, **2** and **3**, affirm the concept that cytokinin activity stems from small-molecule interaction or binding and that this is dependent upon side-chain length and general shape.

EXPERIMENTAL

PMR spectra were recorded on 60 and 100 MHz instruments. Chemical shifts were measured using TMS as an internal standard. Microanalyses were performed by Mr. Joseph Nemeth and his associates. The following compounds have been described previously: 6-(3-methyl-2-butenylthio)purine (**1b**) [8], 6-benzylthiopurine (**2b**) [16], 6-(3-methyl-2-butenyloxy)purine (**1c**) [17], and 6-benzyloxypurine (**2c**) [18]. 6-*Trans-styrylpurine* (**3**) was made by condensation of benzaldehyde with 6-methylpurine in the presence of HCl according to ref. [10], mp 250–251° (reported 246° [10] 248–249 [19]), MS: *m/e* 222 (M^+), 221 (M^+-H), 194 [M^+-(H + HCN)], 167 [M^+-(H + 2HCN)], 140 [M^+-(H + 3HCN)], 128 [$(C_6H_5CH{=}CHCN)$-H].

6-(2-*Phenethyl*)*purine* (**2d**). 6-*trans*-Styrylpurine was hydrogenated over 10% Pd/C for 48 hr at 3 atm to give **2d**, mp 134·5–136° (reported [19] 133–134°).

6-(4-*Methyl*-3-*pentenyl*)*purine* (**1d**). To a suspension of 268 mg (2 mmol) of 6-methylpurine and 0·487 g (4·2 mmol) of N,N,N′,N′-tetramethylethylenediamine in 25 ml of THF stirred under N_2 at 0° was added 2·5 ml (4·2 mmol) of a 15% soln of *n*-butyllithium in hexane. After 30 min, 0·29 ml (2·2 mmol) of 3-methyl-2-butenyl bromide (88%) was added, and stirring was continued for 30 min. The mixture was poured into cold H_2O (25 ml) and the aq. phase washed with Et_2O (2 × 25 ml), neutralized with dil. HCl (to pH 7·15), and extracted with EtOAc (4 × 25 ml). The EtOAc was dried and evaporated *in vacuo*, yielding 190 mg of yellow oil which contained starting material and two faster moving products on TLC ($CHCl_3$–EtOH, 9:1). The major product was isolated by chromatography on Si gel (95 g, $CHCl_3$–EtOH, 9:1), yielding 102 mg (25%) of a colorless oil which crystallized on addition of a few drops of EtOAc. Recrystallization from Et_2O gave an analytical sample, mp 115·5–116·5°. UV: λ_{max} EtOH 267·5 nm (ε 9320), 248·5 sh (6050), λ_{min} 225 (3220); λ_{max} EtOH (0·1 N HCl) 264 (7430), λ_{min} 229 (2900); λ_{max} EtOH (0·1 N NaOH) 272·5 (9650); λ_{min} 240 (2770). PMR ($CDCl_3$) δ = 8·99 (*s*, 1, 2-H), 8·37 (*s*, 1, 8-H), 5·18 (*t*, with additional coupling, 1, *J* 7 Hz, C=CH), 3·12–3·50 (*m*, 2, 6-CH_2), 2·40–2·92 (*m*, 2, allylic CH_2), 1·65 (*s*, 3, Me), 1·57 (*s*, 3, Me). MS: *m/e* 202 (M^+), 187 (M^+-Me), 172 (M^+-2Me), 159 (M^+-C_3H_7), 147 (M^+-C_4H_7), 134 (M^+-C_5H_8), 120 (M^+-C_6H_{10}), 107 [M^+-(C_5H_8 + HCN)], 93 [M^+-(C_6H_{10} + HCN)]. (Found: C, 65·40; H, 6·82; N, 27·39. $C_{11}H_{14}N_4$ requires: C, 65·32; H, 6·98; N, 27·70%).

Bioassay procedures. The determination of cytokinin activities was based on the tobacco assay as described in ref. [20]. The medium contained the mineral salts specified in Table 6, part A, of ref. [20] and the following organic constituents: 30 g/l. sucrose, 10 g/l. Difco agar, 560 μM *myo*-inositol, 11·4 μM IAA, and 1·2 μM thiamine HCl. To facilitate their soln and to avoid possible degradation by heat, the test compounds were dissolved in DMSO, a series of 3-fold dilutions were made, and aliquots then added to the cooling, autoclaved agar media. Final concn of DMSO did not exceed 0·05% by vol, a concentration which does not affect biological activity in this assay [21].

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