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# SYNTHESIS AND CYTOKININ ACTIVITY OF SOME $N^6$-ACYLAMINOPURINES*

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**Key Word Index**—Cytokinins; 6-acylamino purines; $N^6$-benzoyladenosine 5′-phosphate; synthesis; bioassay.

**Abstract**—The preparation and properties of five 6-acylaminopurines (acyl groups; acetyl, propionyl, butyryl, valeryl and benzoyl) and of $N^6$-benzoyladenosine-5′-phosphate are described. In general these substances have good cytokinin activity in the tobacco and soybean tissue culture assays; 6-benzoylaminopurine was almost as effective as 6-benzylaminopurine.

## INTRODUCTION

THERE EXISTS some confusion in the literature regarding the cytokinin activity of 6-acylaminopurines of the general structure R–CONH–purine. Thus Skoog *et al.*[1] refer to ". . . earlier evidence that 6-benzoyl-, 6-furoyl- and 6-succinylaminopurine were inactive, which led to the notion that the mere presence of a polar group in the side chain would eliminate cytokinin activity." Later Skoog and Armstrong wrote,[2] "The early finding in our laboratory that the presence of a carbonyl group in 6-benzoyl- and 6-furoyl-aminopurine rendered them inactive needs to be reinvestigated". In both instances reference is made to a publication by Strong.[3] However, no mention is made in this work of either the synthesis or biological activity of these compounds. By contrast Berridge *et al.*[4] report that 6-benzoylaminopurine (6-benzamidopurine) and 6-butyrylaminopurine were active in a manner similar to that of 6-benzylaminopurine in the chinese-cabbage leaf-disc assay for cytokinins, although no data is presented either for the synthesis or for the activity of these substances.

So far as we are aware the only other studies of cytokinin activity in adenine derivatives containing a carbonyl group in the side chain are those of Rothwell and Wright[5] who reported that a series of alkoxyacylaminopurines had significant cytokinin activity although less than that of kinetin itself, and Dyson *et al.*[6] who demonstrated cytokinin activity in certain analogs of an aminoacyl derivative of adenosine, *N*-(nebularin-6-ylcarbamoyl)-

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*Note added in proof.* DEKHUIJSEN, H. M. and OVEREEM, J. C. have also recently demonstrated cytokinin activity in a series of $N^6$-acylaminopurines [*Phytochem.* **11,** 1669 (1972)].

[1] SKOOG, F., HAMZI, H. Q. and SZWEYKOWSKA, A. M. (1967) *Phytochem.* **6,** 1169.
[2] SKOOG, F. and ARMSTRONG, D. J. (1970) *Ann. Rev. Plant Physiol.* **21,** 359.
[3] STRONG, F. M. (1958) in *Topics in Microbial Chemistry*, pp. 98–157, Wiley, New York.
[4] BERRIDGE, M., RALPH, R. and LETHAM, D. (1970) *Biochem. J.* **119,** 75.
[5] ROTHWELL, R. and WRIGHT, S. T. C. (1967) *Proc. Roy. Soc. Sec.* **167B,** 202.
[6] DYSON, W. H., CHEN, C. M., ALAM, S. N. and HALL, R. H. (1970) *Science* **170,** 328.

threonine, which itself is inactive. There have been no studies dealing with the analogs of the common cytokinins where the methylene side chain has been oxidized to a carbonyl function. In this paper we describe the synthesis and properties of several such compounds and report their biological activity.

## RESULTS AND DISCUSSION

### *Synthesis and Characterization of Test Substances*

6-Benzoylaminopurine (I) was prepared from benzoyl chloride and adenine using the general procedure of Bullock *et al.*[7] Its UV spectrum is similar in many respects to a published spectrum of $N^6$-benzoyladenosine[8] except that there is no marked shift to longer wavelengths under alkaline conditions. A large alkaline shift, however, has been observed in 6-aminoacyl derivatives of adenine.[8,9]

Further evidence for the postulated structure of I comes from its IR spectrum which exhibits strong bands in the 1670–1690 $cm^{-1}$ region and in the 1510–1550 $cm^{-1}$ region. The former is attributed to C=O stretching absorption while the latter is probably an amide N–H bending deformation band.[10] Neither of these occur in the IR spectrum of benzyladenine.[11]

Four other acylated aminopurines were prepared by reacting the appropriate acid anhydride with adenine to give the respective 6-substituted aliphatic derivative. Acetyl-, propionyl-, butyryl- and valeryl-aminopurine were synthesized by this method which is essentially that of Kossel[12] who prepared 6-benzoylaminopurine in this manner. UV spectra of these compounds (II–V) are remarkably similar to one another and have a peak at about 280 nm and lack a pronounced shift under alkaline conditions. In addition, their IR spectra exhibit the characteristic amide peaks at 1670–1690 $cm^{-1}$ and 1510–1530 $cm^{-1}$.

For comparative purposes the 5′-ribonucleotide of I was synthesized by an adaptation of the method of Ralph and Khorana[13] used to synthesize the closely related compound, $N^6$-benzoyldeoxyadenosine-5′-monophosphate; i.e. the reaction of 5′-AMP with benzoyl chloride in pyridine to yield the penta-substituted AMP derivative. Subsequently all except the $N^6$-benzoyl substituent are removed by controlled alkaline hydrolysis, taking advantage of the greater stability in base of the amide linkage of the $N^6$-benzoyl moiety as compared with the $N^1$, 2′, 3′ and 5′ acyl linkages of the remaining benzoyl groups.[13] The UV spectrum of the resulting product, $N^6$-benzoyladenosine-5′-monosphate (IV) is very similar to the deoxy analog.[13]

### *Biological Activity*

Compounds were tested for cytokinin activity in both tobacco and soybean bioassays (see Experimental). Although the data presented in Fig. 1 are for tobacco, a very similar

[7] BULLOCK, M. W., HAND, J. J. and STOKSTAD, E. L. R. (1957) *J. Org. Chem.* **22**, 568.
[8] HALL, R. H. (1964) *Biochemistry* **3**, 769.
[9] HALL, R. H. (1971) in *The Modified Nucleosides in Nucleic Acids*, pp. 43, 109, Columbia University Press, New York.
[10] PASTO, D. J. and JOHNSON, C. R. (1969) in *Organic Structure Determination*, pp. 124–142, Prentice-Hall, Englewood Cliffs, New Jersey.
[11] FOX, J. E., SOOD, C., BUCKWALTER, B. and MCCHESNEY, J. D. (1971) *Plant Physiol.* **47**, 275.
[12] KOSSEL, A. (1889) *Z. Physiol. Chem.* **12**, 241.
[13] RALPH, R. R. and KHORANA, H. G. (1961) *J. Am. Chem. Soc.* **83**, 2926.

pattern of activity was noted when soybean tissue was used as the bioassay. These substances were also tested for their ability to retard chlorophyll disappearance in senescing oat leaves[14] (Table 1).

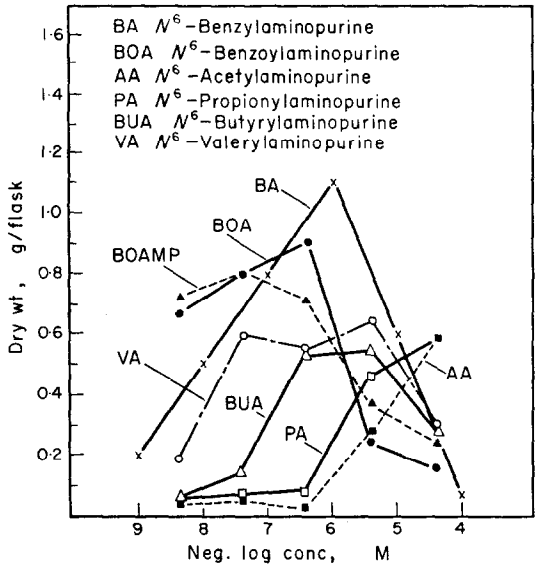


FIG. 1. THE GROWTH OF CYTOKININ REQUIRING TOBACCO TISSUE ON A SERIES OF CYTOKININ ANALOGS. Each point represents the average dry wt of four flasks of tissue grown 3 pieces/flask for 21 days on a standard tissue culture medium (see text).

These results (Figs. 1 and Table 1) show clearly that the presence of a carbonyl group in the side chain of an otherwise active cytokinin does not render it inactive, although the substituent may reduce its effectiveness slightly. Compound I is almost as good a cytokinin

TABLE 1. THE EFFECT OF SOME $N^6$-ACYLAMINOPURINES ON THE SENESCENCE OF DETACHED OAT LEAVES

| Conc. (g/l.) | Test substance | | | | | | |
|---|---|---|---|---|---|---|---|
| | BA | BOA | BOAMP | AA | PA | BUA | VA |
| $1 \times 10^{-6}$ | − | − | − | − | − | − | − |
| $1 \times 10^{-5}$ | − | − | − | − | − | − | − |
| $1 \times 10^{-4}$ | + | − | − | − | − | − | − |
| $1 \times 10^{-3}$ | + | + | − | − | − | − | − |
| $1 \times 10^{-2}$ | + | + | + | − | − | + | + |
| $2{\cdot}5 \times 10^{-2}$ | + | + | + | − | − | + | + |
| $5 \times 10^{-2}$ | + | + | + | − | + | + | + |

Leaves from 2-week-old *Avena sativa* seedlings (var. Forkedeer) which had been grown in subdued light were detached and floated on aqueous solutions of the test compounds in the dark. After 4 days each bioassay set was visually compared with a water control. A + indicates a significant and clearly noticeable maintenance of the chlorophyll content of each of the 5 test leaves as compared to the control. A − indicates generally equivalent chlorophyll disappearance in each lot.

[14] GUNNING, B. and BARKLEY, W. (1963) *Nature, Lond.* **199**, 262.

as 6-benzylaminopurine (BA) at its optimum concentration and may be slightly more effective than BA at lower levels. The ribonucleotide of I is not significantly different than I. Compounds II–V follow the rule observed in their straight chain, unsubstituted, hydrocarbon analogs,[3] i.e. activity is greatest in the 5-carbon chain and decreases with a decrease in chain length. It is possible of course that the cytokinin activity of the compounds described here could be due to their biological reduction to the well-known cytokinin analogs, but in view of the activity displayed by these compounds, the conversion would need to be close to 100%.

## EXPERIMENTAL

*Biological tests.* The origin of the soybean and tobacco tissues used here and their growth on various cytokinin levels have previously been described.[15] The basal medium and culture method have been reported.[16] Test substances were cold-sterilized by filtration. The chlorophyll preservation assay was performed essentially as by Gunning and Barkley.[14]

*Synthesis of test substances. 6-Benzoylaminopurine* (I). 0·5 g adenine was suspended in 10 ml of anh. pyridine to which 1·3 ml of benzoyl chloride (a 20-M excess) was added and the solution heated under reflux for 40 min. The pyridine was then removed *in vacuo*, and the residue dissolved in satd. aq. $NaHCO_3$. To this solution was added 30 ml $CHCl_3$ and the mixture was stored at 0° for 24 hr. The crystals were collected, washed with $H_2O$ at 0° and recrystallized from hot $H_2O$ to give needles (0·464 g, 56·5% yield). For further analysis the material was purified by sublimation (200° at 5 $\mu$) when it had m.p. 238°, uncorr. (previously reported 237·50–238,[12,17] 240–240·5,[7] 241–242,[18] 242–244[19]). The substance gave a single UV absorbing spot in three solvents on TLC: $\lambda_{max}$ 282 nm in $H_2O$, pH 6·0 ($\epsilon$ 17 520). (*Anal.* Calcd. for $C_{12}H_9N_5O$: C, 60·24; H, 3·79; N, 29·27. Found: C, 59·95; H, 3·83; N, 28·44%).

*6-Acetylaminopurine* (II), *6-propionylaminopurine* (III), *6-butyrylaminopurine* (IV) *6-valerylaminopurine* (V). Compounds II–V were prepared by the general method of Kossel.[12] 1·0 $\mu$mol of adenine was suspended in 10 ml of anh. pyridine to which was added 3·0 m-mol of the appropriate acid anhydride. The solution was heated under reflux for 40 min and then worked up as above. Yield and crystallization were similar to those for I. (II) m.p. 216–217° (uncorrected), $\lambda_{max}$ 282 nm, pH 6·0 $H_2O$. (*Anal.* Calcd. for $C_{10}H_{13}N_5O$: C, 54·78; H, 5·95; N, 31·94. Found: C, 54·62; H, 6·27; N, 32·46%). (III) m.p. 221–222°. $\lambda_{max}$ 282 nm, pH 6·0 $H_2O$. (*Anal.* Calcd. for $C_9H_{11}N_5O$: C, 52·68; H, 5·40; N, 34·13. Found: C, 52·32; H, 5·41; N, 34·51%). (IV) m.p. 239–240°. $\lambda_{max}$ 282 nm, pH 6·0 $H_2O$. (*Anal.* Calcd. for $C_8H_9N_5O$: C, 50·52; H, 4·23; N, 36·82. Found: C, 50·36; H, 4·73; N, 37·27%). (V) m.p. Crystals sublimed without melting at about 230°. $\lambda_{max}$ 282 nm, pH 6·0 $H_2O$. (*Anal.* Calcd for $C_7H_7N_5O$: C, 47·46; H, 3·98; N, 39·53. Found: C, 47·34; H, 4·16, N, 39·76%.)

*$N^6$-Benzoyladenosine-5′-monophosphate* (VI). 1·0 m-mol (365 mg) 5′-AMP dried *in vacuo* at 70° for 24 hr was suspended in 25 ml of anh., redistilled pyridine to which was added 20 mmol of freshly redistilled benzoyl chloride. The reaction vessel was tightly stoppered and stirred at 25° in the dark for 4 hr. The dark reddish-brown solution was cooled to 0° and to it was added 50 ml $H_2O$ and 50 ml $CHCl_3$ at 0°. The mixture was stirred for 15 min and then allowed to separate into layers. The $H_2O$ layer was recovered and re-extracted with $CHCl_3$ (2 × 50 ml). The combined $CHCl_3$ extracts containing material presumed to be $N^1$, $N^6$, 2′,3′,5′-pentabenzoyladenosine-5′-monophosphate[13] were evaporated *in vacuo* at 70°. The reddish-brown gum was dissolved in 16 ml pyridine and 8 ml $H_2O$ at 0° to which was then added 24 ml of 2 M NaOH at 0°. The solution was stirred at 0° for 9 min. and then neutralized Dowex 50 cation exchange resin in the $H^+$ form (200–400 mesh) to a pH of 6·5. The resin was filtered off, washed with $H_2O$ and the combined eluates reduced *in vacuo* to 30 ml. Benzoic acid which began to crystallize as the volume was reduced, was extracted with $Et_2O$ (×6) at 0°. The ribonucleotide was recovered as the Ba salt by the addition of a 10-fold excess of $Ba(OH)_4$ followed by the addition of 4 vols. of 95% EtOH. After storage at −20° for 24 hr, the crystals were collected and air dried (224 mg, 47·2% yield). The ribonucleotide was regenerated from its barium salt with $H_2SO_4$ and obtained as a white powder. $\lambda_{max}$ 280 ($\epsilon$ 18 300), $\lambda_{min}$ 243 nm $H_2O$, pH 6·0, $\lambda_{max}$ 288, $\lambda_{min}$ 240 nm HCl, pH 2·0 $\lambda_{max}$ 299, $\lambda_{min}$ 255 nm $NH_4OH$, pH 11·0. The material gave a single UV absorbing spot on TLC in 3 solvents, gave a positive test for organic phosphate,[20] and after being hydrolyzed in 1 N HCl for 1 hr at 100° yielded $N^6$-benzoyladenine.

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