HETEROCYCLES, Vol. 4, No. 6, 1976

Synthesis of a Cytokinin Metabolite

James D. McChesney* and Russell Buchman
Department of Medicinal Chemistry
University of Kansas, Lawrence, Kansas 66045

A number of plant tissues convert approximately 20-25% of the cytokinin, 6-benzylaminopurine, into a stable, long-lived derivative previously identified tentatively as 7-glucofuranosyl-6-benzylaminopurine. This substance has been synthesized by an unambiguous route and shown not to be identical with the long-lived metabolite. Synthesis and comparison of 7-β-glucopyranosyl-6-benzylaminopurine with the metabolite proves that to be the structure of the metabolite instead.

Previous reports from this laboratory[1,2] have described the short term metabolism of the cytokinin, 6-benzylaminopurine (BA) in a number of tissues. It was shown that within 2 hr BA is partially converted to its ribonucleoside (BAR) and its 5' ribonucleotide (BAMP). At about 4 hr a new substance appears and continues to increase in amount so that by 48 hr it accounts for more than 20% of the BA taken up by the tissue. Once having

*To whom correspondence should be directed

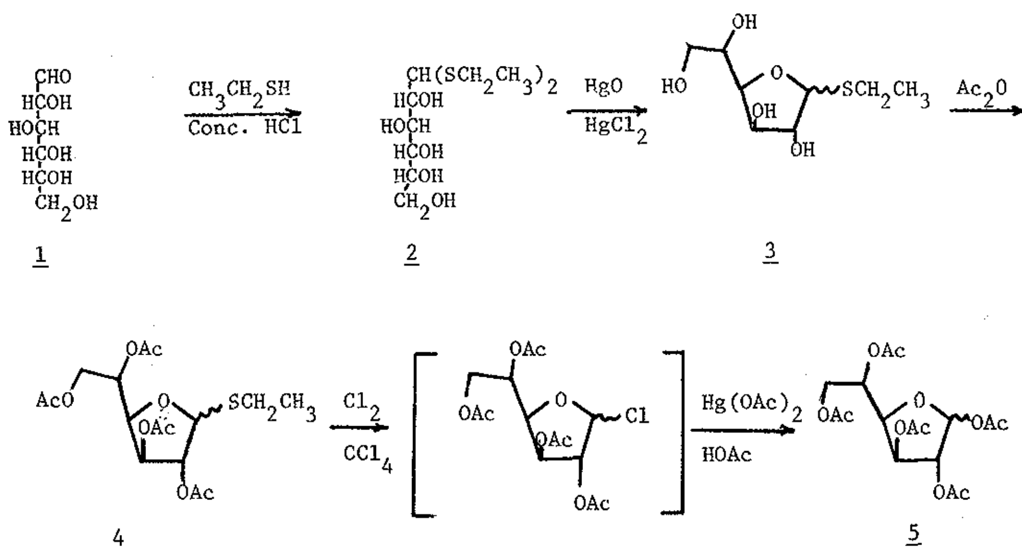
reached this level, the substance is remarkably stable, persisting in the tissue for up to 60 days, while BA, BAR and BAMP rapidly disappear, largely by cleavage of the benzyl side chain. Since the tissue continues to grow long after there is any detectable BA, BAR, or BAMP, it occurred to us that the stable metabolite might be a protected or active form of the cytokinin. The potential importance of such a substance led to its isolation and tentative identification as 7-glucofuranosyl-6-benzylamino-purine.[3] The unusual nature of the proposed metabolite structure required an unambiguous structure identification. We report herein the synthesis of both the 7-β-glucofuranosyl-6-benzylaminopurine and the demonstration that the metabolite is, in fact, 7-β-gluco-pyranosyl-6-benzylaminopurine.

Three approaches to the synthesis of 7-glycosyl adenine derivatives were explored. The first involved attempted direct condensation of the purine chloromercuri derivative with a suitably protected sugar halide by the method of Davoll and Lowy[4]. The yield of 7-substituted-6-benzylaminopurine formed in this way was quite low while the 3 and 9 substituted glycosides predominated[5]. An alternative method for preparation of 7-substitured purines in relatively high yields makes use of a pyrimidinyl formamide[5]. However, attempted base catalyzed condensation of a suitably substituted pyrimidinyl formamide with various sugar halides failed in all cases. Apparently the bulky protected sugar moiety

is not a suitable alkylating agent in this case. A third (and in our hands quite successful) method is that of Townsend and co-workers[7]. That approach involves formation of a suitable purine precursor having the nucleoside bond formed at position 7 early in the sequence.

Preparation of the glucosyl precursors:

The successful preparation of the required glucofuranosyl precursor, 1,2,3,5,6-penta-O-acetyl-D-glucofuranoside (5), was patterned after the procedure of Wolfrom[8] and resulted in an over-all yield of 31%. The sequence is outlined in Scheme 1 (all intermediates gave satisfactory analyses, spectral properties and melting points).



Scheme 1

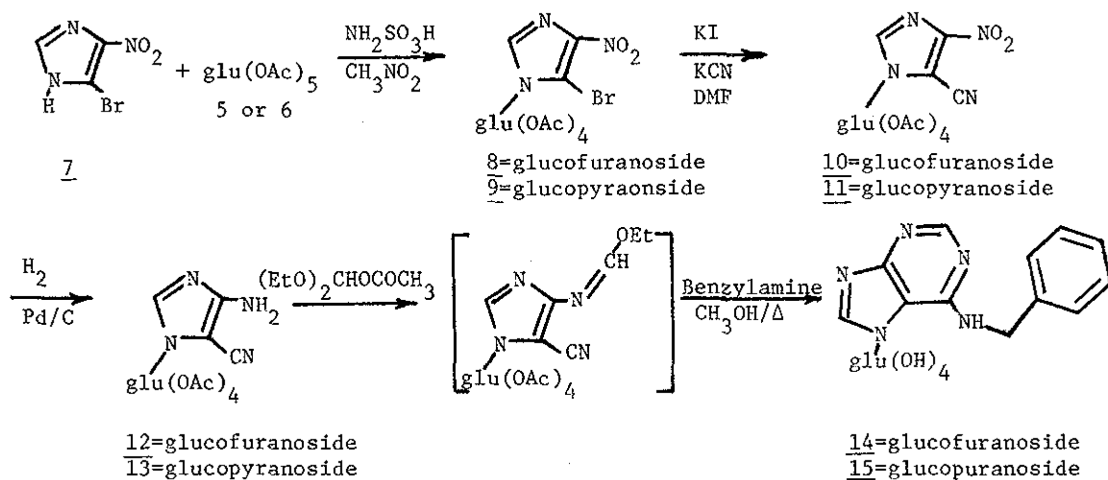
Preparation of 1,2,3,4,6-penta-O-acetyl-β-D glucopyranoside (6) was accomplished in 54% yield by refluxing D-glucose in acetic anhydride-sodium acetate for 20 min[9] [m.p. 131-132° Lit.[9] 132°].

Preparation of the purine precursor:

The required 4-bromo-5-nitroimidazole (7) was prepared in 68% overall yield by the method used by Balaban and Pyman[10] [m.p. 271-272° Lit.[10] 279°].

Glycoside formation and elaboration of 7-glucosyl-6-benzylamino-purines:

The formation of the intermediate imidazole glucoside (8 or 9) by the fusion method of Townsend and co-workers[7] was very low yielding. However, the method of Ishido et. al. employing sulfamic acid in nitromethane was found to be satisfactory, yielding from 25-40% of the desired substituted imidazole glucoside (8 or 9). (See Scheme 2).



Scheme 2

When (8) or (9) was treated with KI and KCN in anhydrous DMF at RT, the cyanonitroimidazole glucoside tetraacetate was formed in 74% yield for the glucopyranoside (11)[analysis, m.p. 158-160°, i.r. (KBr) 2260 cm^{-1} (C≡N)] and glucofuranoside (10) [analysis, syrup, i.r. (KBr) 2250 cm^{-1} (C≡N)]. Catalytic reduction over 10% palladium on charcoal at 50 psi readily afforded the amino-cyanoimidazole glucoside tetraacetates [glucopyranoside, (13) 90% yield, analysis, m.p. 186-87°, i.r. (KBr) 3380, 3460 cm^{-1} (NH_2), 2200 cm^{-1} (C≡N); glucofuranoside (12), 96%, syrup, i.r. (KBr) 3380, 3470 cm^{-1} (NH_2)]. The aminocyanoimidazole glucoside tetraacetates were condensed with diethoxymethylacetate at reflux for 2-4 hr, the excess diethoxymethylacetate removed *in vacuo* and the red syrupy residue dissolved in anhydrous methanol containing a 10 molar excess of benzylamine and refluxed for 15-24 hr. In the case of the glucopyranoside, the methanol solution, upon cooling afforded a solid precipitate which was filtered and recrystallized (H_2O) to afford (55%) 7-glucopyranosyl-6-benzylaminopurine (15). [m.p. 298-299°, analysis, pmr δ (DMSO d_6) 8.28, 8.24 (1 H each, δ, C2H and C_8H purine, 7.81 (1 H, br.s, NH), 7.30 (5 H-m-ArH), eims, $M^{\oplus}$ = 387, u.v. (H_2O) pH 1, λ_{max} 273, ε = 8,600, pH 11, λ_{max} 272, ε = 8,900.] The glycoside linkage was assigned as β based upon the chemical shift or the anomeric carbon signal (CMR) at 87.0 (relative to dioxane) which corresponds to those reported.[12] In the case of the glucofuranoside (14), no precipitate formed upon cooling. The solvent and excess benzylamine were removed *in vacuo* to yield a syrupy residue. The residue was chromatographed (silica gel,

ethylacetate-methanol, 3:1) to afford a homogenous pale yellow amorphous solid (40% yield). [Pmr δ (CH_3OH d_4) 8.33, 8.25 (1 H each, δ, C_2H and C_8H purine), 7.28 (5 H-m- ArH), 5.28 (1H, bs, NH), eims, $M^{\oplus}$ = 387, u.v. (H_2O) pH 1 λ_{max} 281, ε = 7,200; pH 7 λ_{max} 273, ε = 8,700, pH 11 λ_{max} 272, ε = 8,700.] The glycoside linkage was assigned as β based upon the chemical shift (91.9 relative to dioxane) of the anomeric carbon signal in its CMR spectrum.

Comparison of Metabolite and Synthetic 7-glucosides:

The chromatographic behavior of the two synthetic 7-β-glucosides was examined employing HPLC. The system chosen was reverse phase chromatography: Waters Associates Model U6K injector, Model 6000A pump, Model 440 U.V. detector, 30 cm x 4.0 mm ID C_{18} μ bondapak column, 15% CH_3CN in H_2O; 3.0 ml/min as eluent. The glucopyranoside (15) eluted in 10 ml (3.33 min) whereas the glucofuranoside (14) was retained until 17.5 ml (5.83 min). Co-chromatography of radiolabelled metabolite,[1,2,3] collection of fractions and determination of the position of radiolabel demonstrated exact co-elution of the metabolite and 15. In addition, the spectral properties of the metabolite and the glucopyranoside correspond. A previous report[13] by MacLeod and coworkers also demonstrates the metabolite to be different from 7-β-glucofuranosyl-6-benzylaminopurine (14). The structure of the metabolite is clearly established by these experiments. The previously reported biological activity of the metabolite[1] and the lack of biological activity of the synthetic 7-glucopyranoside[5] must now be reexamined.

Acknowledgements.

The authors wish to thank the National Institutes of Health, for financial support of this work under grant numbers 5-K04-GM 00102 and GM 5 101 GM 01341.

REFERENCES

1) J. E. Fox, J. C. Cornette, G. Deleuze, W. Dyson, C. Giersak, P. Niu, J. Zapata, and J. D. McChesney, Plant Physiology, 1903, 52, 627.

2) W. Dyson, J. E. Fox, and J. D. McChesney, Plant Physiology, 1972, 49, 506.

3) G. Deleuze, J. D. McChesney, and J. E. Fox, Biochem. Biophys. Res. Comm., 1972, 48, 1426.

4) J. Dovall and B. A. Lowy, J. Amer. Chem. Soc., 1951, 73, 1650.

5) J. E. Fox and J. D. McChesney, Proc. VIII Int. Conf. Plant Growth Substances, 1973, Hirokawa Pub. Co., Tokyo. pg. 468 (1974).

6. J. Montgomery and K. Hewson, J. Org. Chem., 1961, 26, 4469.

7) R. J. Rousseau, R. P. Panzica, S. M. Reddick, R. K. Robins, and L. B. Towsend, J. Org. Chem., 1970, 35, 631.

8) M. L. Wolfrom and W. Groebke, J. Org. Chem., 1963, 28, 2986.

9) M. L. Wolfrom and A. Thompson, Methods in Carbohydrate Chemistry, 1963, II, 211.

10) I. E. Balaban and F. L. Pyman, J. Chem. Soc., 1922, 121, 947.

11) Y. Ishido, H. Tanaka, T. Yoshino, M. Sekiya, K. Iwabuchi, and T. Sato, Tetrahedron Letters, 1967, 5245.

12) E. Breitmaier and W. Voelter, Tetrahedron, 1973, 29, 227; R.G.S. Ritchie, N. Cyr, B. Korsch, H. J. Koch and A. S. Perlin, Canad. J. Chem., 1975, 53, 1424, we wish to thank Prof. E. Wenkert for obtaining the CMR data.

13) D. E. Cowley, I. D. Jenkins, J. K. Macleod, R. E. Wummons, D. S. Letham, M. M. Wilson, and C. W. Parker, Tetrahedron Letters, 1975, 1015.

Received, 3rd February, 1976