*Phytochemistry*, 1979, Vol. 18, pp. 1055–1056. © Pergamon Press Ltd. Printed in England 0031-9422/79/0601-1055 $02.00

# SHORT REPORTS

# A NEW INTERMEDIATE IN THE SYNTHESIS OF A TRITIATED CYTOKININ WITH HIGH SPECIFIC ACTIVITY

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(*Revised received* 2 *November* 1978)

**Key Word Index**—Tritiated 6-benzylaminopurine; high specific activity cytokinin; *meta*-iodobenzylaminopurine; high affinity cytokinin binding proteins.

## INTRODUCTION

Recently a good deal of attention has been focused on the isolation and identification of receptors for cytokinins in plant tissues [1–7]. Although a highly specific cytokinin binding protein has been isolated from wheat germ ribosomes [3, 6] and post-ribosomal supernatant [6, 7], its study *in situ* is complicated by the presence of a number of low affinity, non-specific binding moieties present in the extracts. Likewise recent attempts in our laboratory to isolate high affinity cytokinin binding moieties demonstrated in pumpkin cotyledons and bean embryos (unpublished results) have met with a similar problem. It appears that the level of high affinity binding sites is low in comparison with non-specific low-affinity binding substances in the plant tissues so far investigated. Ligands of high sp. act. are required to detect specific binding sites under the conditions described above, and to this end Sussman and Firn have described the synthesis of *p*-bromo-6-benzylaminopurine which was used to prepare a specifically tritiated 6-benzylaminopurine ($Bzl^6Ade$) at a sp. act. of 10 Ci/mmol [8]. This compound, a highly active cytokinin, which they generously made available to us, has proved exceedingly useful in studies of cytokinin receptors. We report here the synthesis of a second halogenated intermediate, *m*-iodo-6-benzylaminopurine (*m*-IBA). This compound has the advantage of being more readily hydrogenated than the *p*-bromo analog providing a final product with considerably higher sp. act. In addition the synthesis and purification reported here has several advantages over that previously described [8].

## RESULTS AND DISCUSSION

Our previously reported [9] modification of the Daly and Christensen [10] synthesis of 6-substituted aminopurines was used for the preparation of *m*-IBA. The present modification has considerable advantage over the original procedure in terms of yield and purification of product because of the relative insolubility of the product compared with the reactants. The product, *m*-iodo-6-benzylaminopurine, a previously unreported analog of the halogenated benzyladenines first described by Okumura *et al.* [11], crystallized out of the reaction mixture as fine needles which were collected by centrifuation, washed three times with $H_2O$ at 24° and twice recrystallized from EtOH–$H_2O$ (1:1). These crystals had a mp of 243–244.5° and elemental analysis indicated C, 41.25; H, 2.98; N, 20.10; I, 35.67. $C_{12}H_{10}N_5I$ requires: C, 41.05; H, 2.87; N, 19.94; I, 36.14%.

As described for the *p*-bromo analog [8], the UV spectrum of the compound is identical with that of $Bzl^6Ade$ under a variety of pH conditions. However, the product was clearly separated from $Bzl^6Ade$ and from a potential contaminant, *m*-iodobenzylamine·HCl by TLC on Si gel and by HPLC. The recrystallized product appeared as a single UV absorbing entity in several TLC systems and as a single symmetrical peak on HPLC. Twice recrystallized material was further purified by sublimation for MS. The presumed structure was confirmed via its MS and the presence of a $M^+$ at *m/e* 350 was noted. In addition there were prominent peaks at *m/e* 233, 224, 218, 148, 119, 106, and 91 which corresponded respectively to the iodinated benzylamino cation, the benzyladenine cation, the iodinated tropylium ion, the methyladenine cation, the purine cation, the benzylamine cation and the tropylium ion [12, 13]. The fragmentation pattern of the product is therefore nearly identical with that of BA [12] except for the iodine containing fragments.

Model studies on the dehalogenation of *m*-IBA with $H_2$ gas were done by catalytic hydrogenation in EtOH with palladium on carbon as catalyst at atmospheric pressure. HPLC analysis showed the hydrogenolysis of the iodo derivative to be complete after 6 hr. Radiolabeling was performed on a 35 mg sample of *m*-IBA with carrier free tritium gas (sp. act. 59 Ci/mmol) by the method worked out in the model studies except that the reaction was run for 10 hr. Tritiation and removal of labile tritium was performed by New England Nuclear, 547 Albany Street, Boston, Mass. 02118, U.S.A.

The tritiated sample (*ca* 2 Ci) in 10 ml EtOH was diluted to 1 l. with 95% EtOH. A 100 mCi sample was then repeatedly evaporated *in vacuo* and redissolved in EtOH to remove residual exchangeable tritium and this material was chromatographed by HPLC on a $C_{18}$ reverse phase 10 μ silica, preparative column using 50% aq. MeCN as eluant. The material eluting at the retention vol. of authentic $Bzl^6Ade$ was collected and lyophilized twice in 75% EtOH. Rechromatography by HPLC showed a homogeneous product. UV absorbance was measured at 271 nm using a Cary 118C UV–Vis

* In partial fulfilment of the requirements for the Ph.D. degree.

spectrophotometer and radioactivity determined by liquid scintillation spectrometry. From 3 separate determinations, the sample was found to contain a total of 22.7 ± 0.5 mCi and 8.3 ± 0.5 × $10^{-7}$ mol Bzl$^6$Ade, indicating a radiolabel sp. act. of 27 ± 1 Ci/mmol ($E_{max}$ of 20850 $M^{-1}$).

The new intermediate described in this paper has allowed us to prepare tritiated Bzl$^6$Ade at a sp. act. very near the theoretical maximum of 27 Ci/mmol for a specifically labeled compound substituted with a single tritium atom. This level of activity is considerably higher than any yet reported for a labeled cytokinin and the labeled compound has already proved exceedingly useful in the detection of cytokinin binding sites which exist at low levels in the vegetative parts of a number of plants (data to be published elsewhere). The high sp. act. Bzl$^6$Ade is especially valuable for discriminating between low affinity and high affinity binding moities, particularly where the latter exist in much lower concentrations than the former. Binding studies where the labeled ligand is at concentrations nearly 1 × $10^{-10}$ M can be routinely carried out allowing one to detect low concentrations of high affinity binding moieties. If required by the system, sp. act. up to 3 times higher than that reported here can be achieved for Bzl$^6$ Ade in which the di- or tri-iodo analog is utilized as the starting material, following the same general procedure.

## EXPERIMENTAL

*Synthesis of* m-*IBA.* 6-Chloropurine (500 mg, 3.35 × $10^{-3}$ mol) was refluxed together with 500 mg (1.8 × $10^{-3}$ mol) of *m*-iodobenzylamine·HCl (97%) (Aldrich Chemical Co.) for 6 hr in 25 ml of 50 mM Na Pi buffer, pH 7.8. Crystalline material began settling out of soln after 30 min of reflux and continued for the duration of the reaction. The crystals were collected by centrifugation, washed × 3 with $H_2O$ at 4° and air-dried. About 400 mg of product was obtained. Although no attempt was made to recover quantitatively the product from the reaction mixture, we estimate the yield at this stage at 70% or better. Final purification was achieved by 2 recrystallizations from EtOH–$H_2O$ (1:1). A small sample was subsequently sublimed for MS.

*Acknowledgements*—This research was supported by grants from the National Institutes of Health (GM-22842) and The National Science Foundation (PCM76-15250). J. Erion was supported for part of this work by a Graduate Fellowship from the University of Kansas.

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