p-[^{3}H]-*m*-Azidophenyl Acetic Acid, a Useful Reagent for the Synthesis of Radioactive Photoaffinity Ligands. Synthesis of Photoaffinity Labelling Ecdysones

Marcus F. Boehm,[a] Koji Nakanishi*[a] and Peter Cherbas[b]

[a] *Department of Chemistry, Columbia University, New York, NY 10027, USA*
[b] *Department of Biology, Indiana University, Bloomington, IN 47405, USA*

The synthesis of [^{3}H]-*p*-azidophenyl acetic acid and its use in the preparation of aryl azido photoaffinity labelled 20-hydroxyecdysone analogues is described.

20-Hydroxyecdysone **1** is the steroid moulting hormone of arthropods and, like other steroids, is a regulator of gene expression.[1–3] Although the molecular biology of its action is being studied in numerous laboratories,[4–6] details of the ecdysone–receptor interaction are largely unknown. The synthesis of high affinity radiolabelled hormone analogues such as [^{125}I]-26-iodoponasterone A have facilitated detection of ecdysone receptors.[7,8] We are now attempting to utilize radioactive photoaffinity labelling probes for receptor isolation and furthermore, to characterize the steroid binding site.

Radiolabelled photoaffinity ligands are light-activatable moieties that form reactive intermediates, *e.g.* carbenes and nitrenes, which insert into the amino acids of a receptor site and radioactively tag the receptor for isolation and structural studies. Several photolabile functionalities are widely utilized including diazoketones, diazoacetates, aziridines and aromatic azides.[9] Of these moieties, the aromatic azides containing ^{3}H or ^{125}I are synthetically the most readily incorporated in biologically active molecules where they form a reactive nitrene upon irradiation with 254 nm light. Although the specific activity of tritium is *ca.* 10–100 times lower than ^{125}I, the use of tritium as a radiolabel is often preferable to ^{125}I because of its longer halflife (12.3 years for tritium *vs.* 60 days for ^{125}I); also, loss of iodine and subsequent non-specific labelling with free iodine is often associated with the photolysis of iodinated molecules.[10–12]

We report the synthesis of two [^{3}H]-photoaffinity labelling ecdysteroid analogues, 2-(*p*-azidophenylaceto)-20-hydroxyecdysone (APA-20-hydroxyecdysone) **2b** and 26-(*p*-azidophenylaceto)-inokosterone (APA-inokosterone) **3b** which competitively bind with the ecdysteroid receptor. We also describe a three step synthesis of *p*-azido-*m*-[^{3}H]-phenyl acetic acid (APAA) **4b**[13] from *p*-aminophenyl-acetic acid **5a** which can be coupled with nucleophiles (alcohols and amines), and its application to the photoaffinity labelling of ecdysone ligands. The *p*-azidophenyl acetic acid moiety was chosen

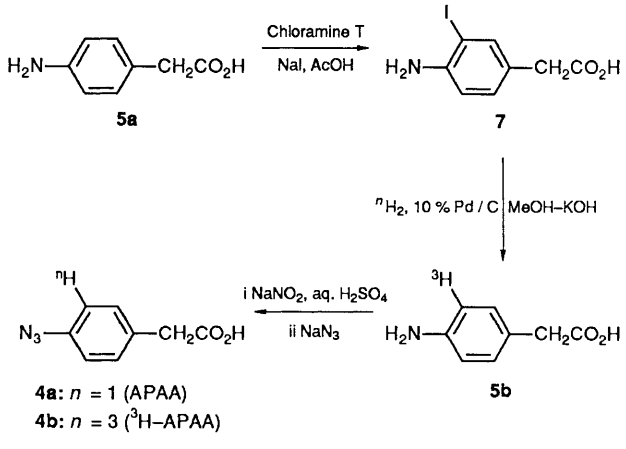

Fig. 1

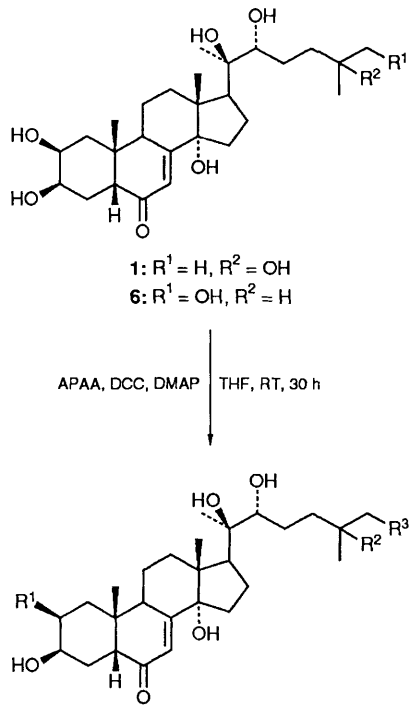

Fig. 2

because initial attempts to perform selective dicyclohexyl carbodiimide (DCC) coupling of *p*-azido benzoic acid with a hindered primary alcohol such as that found at C-26 of inokosterone **6**, resulted in no reaction, presumably because of the low reactivity of the benzoic acid. *p*-Azidophenyl acetic acid is more reactive than *p*-azidobenzoic acid, readily coupling with ecdysteroids and is proving to be useful in other systems containing free hydroxy's and/or amines (unpublished results).

p-Aminophenyl acetic acid **5a** was treated with chloramine T and NaI in acetic acid to give *p*-amino-*m*-iodophenyl acetic acid **7** in 41% yield (Fig. 1).[14,15] Catalytic tritiation of *p*-amino-*m*-iodophenyl acetic acid in MeOH–KOH with 10% Pd/C and carrier-free $^3H_2$ resulted in *p*-amino-*m*-^{3}H-phenyl acetic acid **5b** with specific activity of 19 Ci/mmol.† The phenyl amines **5a** and **5b** were oxidized with $NaNO_2$ in aqueous H_2SO_4, and treated with NaN_3 to give *p*-azido-*m*-^{n}H-phenyl acetic acids (APAA) **4a** (n = 1) and (^{3}H-APAA) **4b** (n = 3) (89% yield, specific activity 19 Ci/mmol), UV (MeOH) λ_{max} = 252 nm, ε = 12,000.[13,14] A mixture of 20-hydroxyecdysone **1** and inokosterone **6**‡[16,17] (ratio = 1.3:1), extracted from the root of *Achyranthes fauriei*,[18,19] was treated with 1.5 equiv. each of *p*-APAA **4a**, DCC and dimethylaminopyridine in dry THF for 30 h to give a 1:1 mixture of 2-(*p*-APA)-20-hydroxyecdysone **2a** and 26-(*p*-APA)-inokosterone **3a** in approximately 35% combined yield after HPLC[16] purification (Fig. 2): HRFAB–MS 640.3578, calcd. for $C_{35}H_{50}N_3O_8$ 640.3597; UV (MeOH) λ_{max} = 250 nm, ε = 18000.§ Similarly, the radiolabelled ecdysone analogues were synthesized from ^{3}H-APAA **4b** to give ecdysteroids **2b** and **3b** at specific activity > 6 Ci/mmol. The products coeluted with authentic samples of ecdysteroids **2a** and **3a**.

Preliminary biological tests were designed to test competition by the azidophenyl ecdysone analogues (in the absence of light) for specific [^{125}I]-26-ponasterone A binding in a Kc cell extract.[7] At 1 μmol dm^{-3} concentration, both analogues compete poorly. However, after photolysis (254 nm), each reduced specific binding of the radioligand by 20–40%. Further photoaffinity studies are ongoing.

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† Catalytic tritiation was performed by Amersham using carrier-free tritium gas.

‡ Inokosterone 6 is a mixture of C-25 epimers.

§ *Selected spectroscopic data* for: **2a** ^{1}H NMR ([$^2H_6$]MeOH) δ 0.79 (s, 13-CH_3), 0.89 (s, 10-CH_3), 1.09 [s, 25-(CH_3)$_2$], 1.10 (s, 20-CH_3), 2.31 (dd, *J* 9.1 and 4.4 Hz, 5-C*H*), 3.20 (m, 9-C*H*), 3.58 (s, Ph-CH_2), 4.01 (m, 3-C*H*), 4.89 (dt, *J* 14.8, 3.8 and 3.8 Hz, 2-C*H*), 5.72 (d, *J* 2 Hz, 7-CH), 6.93 (d, *J* 8.5 Hz, Ph-*H*), 7.24 (d, *J* 8.5 Hz, Ph-*H*).

3a ^{1}H NMR ([$^2H_6$]MeOH) δ 0.79 (s, 13-CH_3), 0.82 (d, *J* 6.9 Hz, 25-CH_3), 0.87 (s, 10-CH_3), 1.07 (s, 20-CH_3), 2.27 (dd, *J* 11.7 and 5.7 Hz, 5-C*H*), 3.06 (m, 9-C*H*), 3.55 (s, Ph-CH_2), 3.73 (dt, *J* 12.1, 3.7 and 4.1 Hz, 2-C*H*), 3.85 (m, 3-C*H* + 26-CH_2), 5.71 (d, *J* 2.0 Hz, 7-C*H*), 6.93 (d, *J* 8.4 Hz, Ph-*H*), 7.22 (d, *J* 8.4 Hz, Ph-*H*).