

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

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The synthesis of [³H]-*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 [¹²⁵I]-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 ³H or ¹²⁵I 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 ¹²⁵I, the use of tritium as a radiolabel is often preferable to ¹²⁵I because of its longer half-life (12.3 years for tritium *vs.* 60 days for ¹²⁵I); 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 [³H]-photoaffinity labelling ecdysteroid analogues, 2-(*p*-azidophenylaceto)-20-hydroxy-

ecdysone (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*-[³H]-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

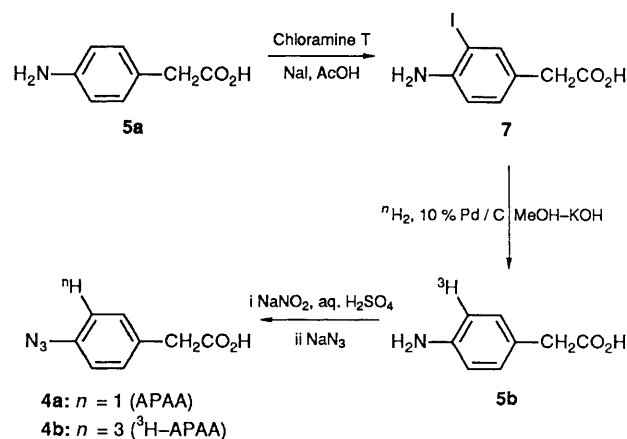


Fig. 1

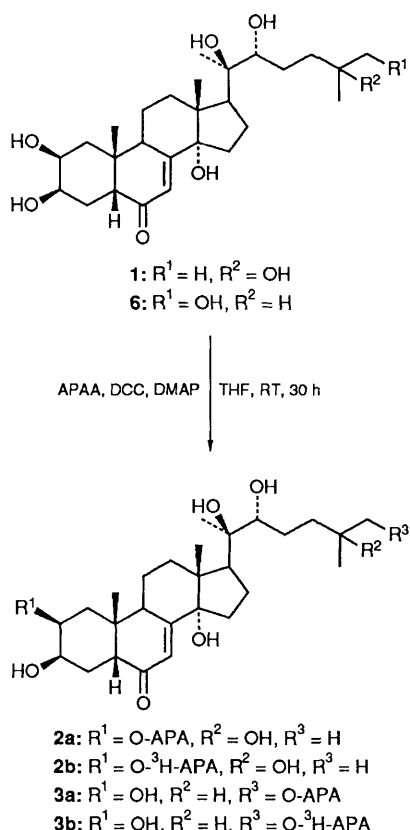


Fig. 2

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 ³H₂ resulted in *p*-amino-*m*-³H-phenyl acetic acid 5b with specific activity of 19 Ci/mmol.[†] The phenyl amines 5a and 5b were oxidized with NaNO₂ in aqueous H₂SO₄, and treated with NaN₃ to give *p*-azido-*m*-ⁿH-phenyl acetic acids (APAA) 4a (*n* = 1) and (³H-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[‡] (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 HPLC¹⁶ purification (Fig. 2): HRFAB-MS 640.3578, calcd. for C₃₅H₅₀N₃O₈

640.3597; UV (MeOH) λ_{max} = 250 nm, ε = 18000. § Similarly, the radiolabelled ecdysone analogues were synthesized from ³H-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 [¹²⁵I]-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.

We are grateful to Dr. K. Nomoto, Suntory Institute for Biorganic Research, Osaka, for providing us with *Achyranthes fauriei*. The studies were supported by National Institutes of Health awards AI 10187 (K. N.) and GM 13552 (M. F. B.).

Received, 30th August 1990; Com. 0/03927F

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§ Selected spectroscopic data for: 2a ¹H NMR ([²H₆]MeOH) δ 0.79 (s, 13-CH₃), 0.89 (s, 10-CH₃), 1.09 [s, 25-(CH₃)₂], 1.10 (s, 20-CH₃), 2.31 (dd, *J* 9.1 and 4.4 Hz, 5-CH), 3.20 (m, 9-CH), 3.58 (s, Ph-CH₂), 4.01 (m, 3-CH), 4.89 (dt, *J* 14.8, 3.8 and 3.8 Hz, 2-CH), 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 ¹H NMR ([²H₆]MeOH) δ 0.79 (s, 13-CH₃), 0.82 (d, *J* 6.9 Hz, 25-CH₃), 0.87 (s, 10-CH₃), 1.07 (s, 20-CH₃), 2.27 (dd, *J* 11.7 and 5.7 Hz, 5-CH), 3.06 (m, 9-CH), 3.55 (s, Ph-CH₂), 3.73 (dt, *J* 12.1, 3.7 and 4.1 Hz, 2-CH), 3.85 (m, 3-CH + 26-CH₂), 5.71 (d, *J* 2.0 Hz, 7-CH), 6.93 (d, *J* 8.4 Hz, Ph-H), 7.22 (d, *J* 8.4 Hz, Ph-H).

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

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