Scientia Pharmazeutica (Sci. Pharm.) **69**, 161–166 (2001)

Reaction of Prop-2-ynylamine with Isochromadiones : Formation of Amides.

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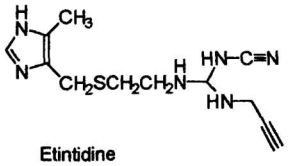
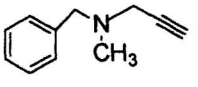
Key Word : Isochromadiones, Prop-2-ynylamine, Amide.

Abstract
The ring opening of isochromadiones by prop-2-ynylamine was accomplished in non-polar solvents to form N-prop-2-ynylbenzamide-2-acetic acid **3** N-prop-2-ynylhomophthalimide **4**. Compound **3** was found out to be an intermediate product in the formation of **4.** These compounds were screened for anticonvulsant and antibacterial properties and were found to have no activity.

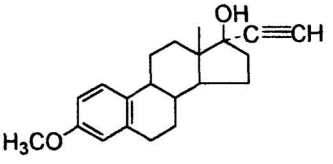
Introduction
Isochromadione have been used to prepare various heterocyclic compounds notably isoquinolones which have been of importance in pharmaceutical and chemical industries[1,2]. The importance of prop-2-ynyl moiety in some biologically useful agents cannot be over-emphasised .



Etintidine



Pargyline



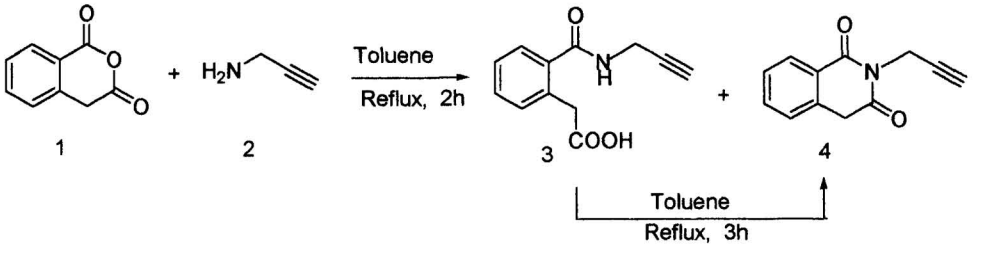
Mestranol

Etintidine is a H_2 antagonist and has been employed as an anti-ulcer drug[3] while pargyline is a monoamine oxidase inhibitor[4]. Mestranol is a synthetic oral contraceptive.[5] Recent literature search revealed that ring opening of 3-azaisatoic anhydride with acetylenic amine gave the corresponding nicotinamides[6] and the reaction of N-methylisatoic anhydride with acetylenic amines yielded acetylenic amides[7]. It is well established that the reaction between isatoic anhydride and acetylenic amines gave benzamides, which on treatment with triphosgene in pyridine cyclocondensed to the corresponding quinazolinones[8]. There has been renewed interest in the pharmacological activity of substituted benzamides demonstrated by the research activities of Clark [9,10,11]

and co-workers. The anticonvulsant activity of some of these substituted benzamides stimulated the investigation of the reaction of isochromadione with prop-2-ynylamine leading to the formation of amides.

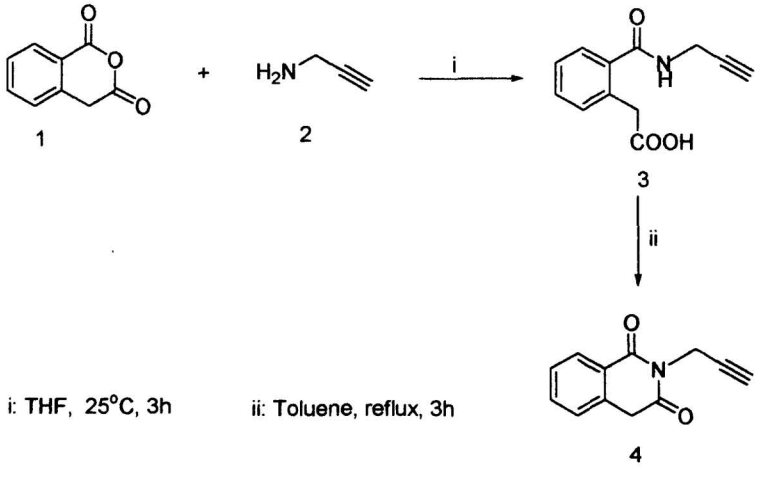
Discussion

We initially were interested in the synthesis of the target compound N-prop-2-ynylhomophthalimide **4** and proceeded by refluxing a mixture of **1** and **2** in toluene for 2h and monitoring the reaction by thin layer chromatography. Two spots on thin layer chromatography apart from the starting material were observed. The isolation and identification of the two spots revealed them to be **3** and **4**. We reasoned that **3** was probably an intermediate product in the formation of **4**.



1 2 3 4

Further boiling of **3** in toluene for another 3h clearly led to formation of **4**. It became rational to reflux **1** and **2** in toluene for over 4h and determine if **4** will be ultimately produced. After refluxing in toluene for 5h, thin layer chromatography revealed total disappearance of **1** and formation of one spot which was identified and characterised as **4**. The yield was rather low hence the synthetic procedure to **4** was optimised. This was executed by stirring **2** with **1** in tetrahydrofuran at room temperature for 3h. The removal of tetrahydrofuran and subsequent reflux of the residuein toluene for 3h gave **4** in good yield. The compounds were characterised by their melting points, spectroscopic data and elemental analyses.



1 2 3 4

i: THF, 25°C, 3h ii: Toluene, reflux, 3h

Biological Activities

The compounds were tested for anticonvulsant activity by using Maximal Electroschock Seizure (MES), the Subcutaneous Pentylenetetrazole Seizure threshold test (ScMet) and toxicity in the rotorod test and were found to have no activity and were not toxic at the concentrations used. The antibacterial activity was also performed on the compounds using cultured organisms like *Staphylococcus aureus* and *Escherichia coli* and the result shows that the compounds had no antibacterial activity.

Table 1: Anticonvulsant activity in the MES test and the ScMet test and toxicity in the rotorod test of **3** and **4** following intraperitoneal administration to mice. The numbers are expressed as animals protected/animals tested

		MES		ScMet		Toxicity		
Compound	Dose [mg/kg]	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	Class [a]
3	30	0/1	0/1	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	0/1	0/1	0/1	0/1	0/4	0/2	
4	30	0/1	0/1	0/1	0/1	0/4	0/2	3
	100	0/3	0/3	0/1	0/1	0/8	0/4	
	300	0/1	0/1	0/1	0/1	0/4	0/2	

[a]The classification is as follows:
1 = anticonvulsant activity at 100mg/kg
2 = anticonvulsant activity at doses greater than 100mg/kg
3 = Compound inactive at 300mg/kg.

Antimicrobial Screening

Table 2: *Staphylococcus aureus* (NCTC 6571)

Compound used (Dose/Concentration = 200μg)	Zone of inhibition (After 24hrs)
N-Prop-2-ynylbenzamide- 2-acetic acid (**3**)	None
N-Prop-2-ynylhomophthalimide (**4**)	None
Ampicillin	18 mm
DMSO (0.2ml)	None

Table 3: *Escherichia coli* (NCTC 10418)

Compound used (Dose/Concentration = 200μg)	Zone of inhibition (After 24hrs)
N-Prop-2-ynylbenzamide- 2-acetic acid (**3**)	None
N-Prop-2-ynylhomophthalimide (**4**)	None
Ampicillin	22 mm
DMSO (0.2ml)	None

Experimental

Melting points were determined with a Kofler hot stage microscope and were uncorrected. The reactions and purity of the products were monitored by thin-layer-chromatography using pre-coated silica gel plates (Merck $60F_{254}$). Silica gel Merck 60 (70-230 mesh) was used for column chromatography. NMR (^1H and ^{13}C) were recorded on a Varian Gemini 200 (TMS), IR were measured on a Perkin-Elmer type 457 and the MS were determined using Varian MAT 44S, EI: 70 eV.

N-Prop-2-ynylbenzamide-2-acetic acid (3) and N-prop-2-ynylhomophthalimide (4)

To isochromadione (0.40g, 2.50 mmol) in 30 ml of toluene was added prop-2-ynylamine (0.16g, 3.0mmol) and stirred at reflux for 2h. Column chromatography of the crude product using dichlomethane was carried out to separate the products **3** and **4** from the isochromadione. N-Prop-2-ynylhomophthalimide **4** (0.075g) was isolated first and later N-prop-2-ynylbenzamide-2-acetic acid **3** (0.11g) in 15% and 20% yields respectively.

N-Prop-2-ynylbenzamide-2-acetic acid (3)

To isochromadione (0.70g, 4.33 mmol) in 20 ml of tetrahydrofuran (THF) was added prop-2-ynylamine (0.28g, 5.19 mmol) and stirred at room temperature for 3 h. Solvent was removed and column chromatography (dichloromethane) was used to purify the compound. Crystallization from ethanol gave N-prop-2-ynylbenzamide-2-acetic acid **3** as fine needles. 0.94g (70%); m.p 137-138^{0}C; IR (KBr): 3290 (NH, C≡CH), 3100 (OH), 1697 (C=O), 1632 (C=O), 1600 (C=C); ^1H-NMR (200 MHz) (acetone d_6): δ = 2.70 (d, 1H, ≡CH), 3.99-4.03 (brs, 4H, 2xCH_2), 7.44 (m, 2H, Ar-H), 7.53 (d, 1H, Ar-H), 7.81 (brs, 1H, NH), 7.97 (d, 1H, Ar-H); ^{13}C-NMR (50 MHz acetone d_6): δ = 41.18 (CH_2C≡CH), 71.74 (CH_2C≡CH), 80.61 (CH_2C≡CH), 110.01 (CH_2), 127.44, 131.23, 131.56, 132.17, 132.35, 136.93 (aromatic carbons) 168.66 (C=O) 171.00 (C=O); MS (70eV) m/z: 217 [M^+](2%), 136 (100); $C_{12}H_{11}NO_3$ (217.287); Anal. Calc. C 66.33, H 5.10, N 6.45; Found: C 66.20, H 5.00, N 6.40.

N-Prop-2-ynylhomophthalimide (4)

(i) To isochromadione (0.20g, 1.25 mmol) in 20 ml of toluene was added prop-2-ynylamine (0.08g, 1.51mmol) and stirred at reflux for 5h. After column chromatography (dichloromethane) has been used to purify the compound, crystallization from ethanol afforded N-prop-2-ynylhomophthalimide **4**. 0.05g (20%), mp 169-170^{0}C; IR (KBr): 3245 (-C≡CH), 2100 (C≡C), 1703 (C=O), 1663 (C=O), 1603 (C=C). ^1H-NMR (200 MHz, DMSO d_6): δ = 3.09 (t, 1H, ≡CH), 4.20 (s, 2H, -CH_2), 4.56(d, 2H, N-CH_2-), 7.36–7.47 (m, 2H, Ar-H), 7.66–7.69 (t, 1H, Ar-H), 8.02-8.06 (d, 1H, Ar-H); ^{13}C-NMR (50 MHz, DMSO d_6): δ = 29.56 (CH_2C≡CH), 36.87 (C-4), 73.78 (CH_2C≡CH) 80.21 (CH_2C≡CH), 125.37, 128.30, 128.62, 128.95, 134.74, 136.25 (aromatic carbons), 164.66 (C=O), 170.13 (C=O); MS (70eV) m/z = 199 [M^+] (21%) 171(100).; $C_{12}H_9NO_2$ (199.199) Anal. Calc. C 72.36, H 4.55, N 7.03; Found: C 72.32, H 4.50, N 6.96.

(ii) N-Prop-2-ynylbenzamide-2-acetic acid **3** (0.50g, 2.3 mmol) was refluxed in 20 ml of toluene for 3h. After column chromatography (dichloromethane) has been used to purify the compound, N-prop-2-ynylhomophthalimide **4**. 0.27g (60%) was crystallized from ethanol as needles.

Anticonvulsant testing

Anticonvulsant testing was provided by the Antiepileptic Drug Development Programme, Epilepsy Branch, Division of Convulsive, Developmental and Neuromuscular Disorders, National Institutes of Health, according to standard procedures [12] and included the MES test and the seizure scMet test. In the MES test, an electrical stimulus of 50 mA was delivered for 0.2 sec via corneal electrodes to mal CF1 mice at 30 min and 4 h after the administration of the compounds. Blockade of the tonic extension of the hind limbs was considered protection against seizures. For the scMet test a convulsant dose of 85 mg/kg of pentylenetetrazole dissolved in saline was injected in a loose fold of skin on the back of the neck and the animals were isolated and observed for 30 min. Absence of clonic spasms for at least 5 sec indicated the elevation of the pentylenetetrazole-induced seizure threshold. The acute neurological toxicity was determined in the rotorod test where the animal was placed on a rod rotating at 6 rpm. Neurological deficiency was indicated by inability to maintain equilibrium for 1 min in each of 3 trials. For all these evaluations the compounds were dissolved or suspended in 0.5 % aqueous methyl cellulose.

Antimicrobial Test

25mls of molten nutrient agar were aseptically poured into each of the two sterile petri-dishes and this was allowed to solidify. The cultured organism *Escherichia coli* (NCTC 10418) and *Staphylococcus aureus* (NCTC 6571) were used to flood each of the two nutrient agar plates and the excess was poured away after some time. Using a sterile cork borer, four wells were made on each of the nutrient agar plates. Into each of these wells, 0.1ml of molten nutrient agar was poured in order to seal the base. Using a pipette, 0.2ml (200μg) of 1mg/ml in DMSO of compounds **3, 4**, ampicillin and 0.2ml of DMSO were aseptically introduced into each of the wells respectively. It was allowed to stand on the bench for 30–40 minutes and incubated at 37°C for 24 hours.

Acknowledgement

The authors appreciate the assistance of Department of Pharmaceutical Chemistry, University of Münster, Germany in running the spectra and Dr James Stables, National Institute of Health, Bethseda, USA in the testing of the compounds through the ADD programme.

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Received December 5th, 2000
Accepted March 15th, 2001