ANTIMICROBIAL AGENTS FROM HIGHER PLANTS. NEW SYNTHESIS AND BIOACTIVITY OF TRYPTANTHRIN (INDOLO-[2,1-b]-QUINAZOLIN-6,12-DIONE) AND ITS ANALOGUES.†

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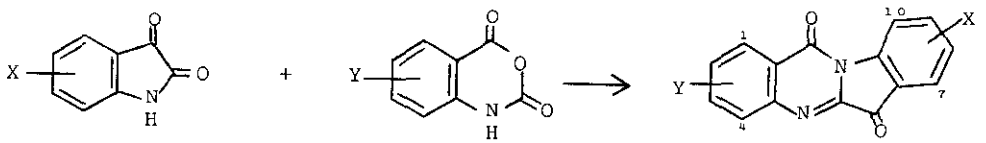
Abstract - Base-catalyzed condensation of substituted isatins and substituted isatoic anhydrides provides a convenient, one-step flexible synthesis of indolo-[2,1-b]-quinazolin-6,12-diones, including the antifungal natural product tryptanthrin. This new process provides the basis for a systematic exploration of the relationship between structure and antimicrobial activity. Preliminary findings demonstrate that non-symmetrically substituted analogues are easily prepared and the method tolerates the presence of a variety of ring substituents.

Strobilanthes Cusia, used in Taiwanese indigenous medical systems for the treatment of dermatophyte infections, such as athletes foot, has as its active principle, tryptanthrin (3) (X = Y = H).[1,2] Remarkably, tryptanthrin also occurs in the unrelated species, Polygonum tinctorium and Isatis tinctoria,[3] and it can also be produced by directed biosynthesis when the yeast, Candida lipolytica, is fed with relatively large quantities of tryptophane.[4] Feeding substituted tryptophane analogues to the culture leads to the production of some "unnatural" analogues, some of which retain bioactivity.[4,5] None of these processes is convenient and they are limited with respect to the sorts of analogues which can be produced. Three syntheses of tryptanthrin have been reported.[6-8] These are limited in scope and give poor yields. In order to make further progress in elucidating the

†Dedicated to Professor Tetsuji Kametani on the occasion of his retirement.

structure-activity relationships in this novel class of antifungal agents, a more flexible synthesis seemed called for-particularly one which allows the convenient preparation of unsymmetrically substituted analogues. We report herein some of our early experiences with what appears to be such a synthesis along with the chemical and biological properties of some new tryptanthrin analogues selected to illustrate the potential power and scope of the new method.

Given the convenient availability of isatins (1) and isatoic anhydrides (2), it appeared that a successful base-catalyzed condensation patterned loosely after our work in the quinolone alkaloid[9] and antibiotic[10,11] areas would have the desired characteristics.



(1) (2) (3)

This turned out to be correct. Under a flow of dry nitrogen, 10.0 g (68mM) of isatin (1, X = H) in 500 ml dry DMF was added over a 15 min. period to 1.64 g (68.3mM) of NaH while stirring. To the resulting deep purple liquid was added 11.55 g of isatoic anhydride in 500 ml dry DMF, with ice cooling, over a 30 min period. The reaction was stirred at room temperature overnight and quenched with 15 ml of MeOH. The resulting mixture was diluted with two volumes of $CHCl_3$ and washed once with water. The aqueous layer was extracted three times with chloroform and the combined and dried (Na_2SO_4) organic layers were concentrated. The first crop of crystalline trypthanthrin which separated consisted of 2.77 g m. p. 263.5-265.5°C. A second crop, 13.54 g, m. p. 230-240 required two crystallizations from chf/EtOAc (2:1) before giving 8.43 g, m. p. 263-264°C (combined yield, 66%): ir(KBr) 1725 and 1680 cm^{-1}; pmr($CDCl_3$) 7.15-8.55δ (m); eims m/z 248(M^+=100%), 220(36%), 192(22%), 124($M^+/2z$, 13%). Anal. Found: C, 72.70; H, 3.33; N, 11.38.

The other analogues were produced essentially in the same manner with the exception that t-BuOK was used as the base for the nitro derivatives. The

results are summarized in the table. The yields are those obtained by direct crystallization and could, in principle, be improved by chromatography of the mother liquors. They were, however, adequate for testing purposes and the simplicity of the method is its main recommendation. The full paper will contain a discussion of the side products and a much more extensive list of analogues.

The previous work of Zähner et al.[4] established that substitution of the hydrogen at positions 2, 4 and 10 in tryptanthrin was beneficial to bioactivity, replacement at position 8 was of variable effect, and that changes at positions 3 and 7 were deleterious. Positions 1 and 9 were virtually unexplored so we concentrated our first efforts here. With the exceptions of 1-bromotryptanthrin (7) and 1-bromo-8-nitrotryptanthrin (11), our data show that changes at these positions are generally deleterious.

A systematic study of the effect of substituent variation by Hansch techniques, as modified by Topliss-Martin,[9] is underway and will be reported in due course.

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Table. Properties of Tryptanthrin and Analogues.*

Compd.	X	Y	Yield	m.p.	Potency**
(3)	H	H	66%	263.5-265.5	(1)50(4)100(5)6.25(6)100
(4)	H	1-Cl	36	320-322	i
(5)	9-Cl	H	63	282.5-283.5	i
(6)	9-Cl	1-Cl	29	341.5-343.5	i
(7)	H	1-Br	35	314-316	(1)25(4)25(5)25(6)50
(8)	H	1-NO_2	26	348-350	i
(9)	8-NO_2	H	9	302-304	(1)12(5)12
(10)	8-NO_2	1-Cl	52	293-294	i
(11)	8-NO_2	1-Br	31	294-296	(1)6.25(5)25(6)50

*All compounds listed gave satisfactory microanalyses for C,H,N and satisfactory mass spectra.

**Expressed as mcg/ml via agar-dilution, streak against the following organisms: (1) Staphylococcus aureus ATCC 13709

(2) Escherichia coli ATCC 9637

(3) Salmonella gallinarum ATCC 9184

(4) Klebsiella pneumoniae AD ATCC 10031

(5) Mycobacterium smegmatis ATCC 607

(6) Candida albicans ATCC 10231

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