# THE STRUCTURE AND PROPERTIES OF SOME INDOLIC CONSTITUENTS IN COUROUPITA GUIANENSIS Aubl.

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Abstract Extraction of the dried fruits from the cannon ball tree, *Couroupita quianensis* Aubl., yielded 6,12dihydro-6,12-dioxoindolo[2,1-b]quinazoline (tryptanthrin), 1a as well as indigo (7) indirubin (8a) and isatin. Compound 1a could readily be synthesized by condensation of isatin with isatoic anhydride (6) in pyridine.

Recently a yellow compound isolated from the fruits of the cannon ball tree, *Couroupita quianensis* Aubl., was identified by us<sup>1</sup> as 6,12-dihydro-6,12-dioxoindolo-[2,1-b]quinazoline (1a) (tryptanthrin), which earlier<sup>2</sup> had been assigned the erroneous formula 2. Subsequently Honda *et al.*<sup>3, 5</sup> identified tryptanthrin as the active principle in the leaves of *Strobilanthes cusia* O. Kuntze, which has a long tradition in Okinawa as a remedy against dermatophytic infections, notably athlete's foot.

Furthermore the Japanese workers could isolate tryptanthrin from *Polygonum tinctorium* and *Isatis tinctoria*. Interestingly 1a can also be produced by directed biosynthesis when the yeast, *Candida lipolytica*, is fed with relatively large amounts of tryptophan.<sup>6 8</sup> The fact that tryptanthrin is a highly specific antimicrobial agent against dermatophytes, notably an effect similar to that of griseofulvin (3) against *Trichophyton mentagrophytes*, has already triggered the development of several syntheses<sup>9 11</sup> as well as cultivation methods,<sup>6 8</sup> of tryptanthrin and various halogen and NO<sub>2</sub>-substituted analogues.

### Structure and synthesis of tryptanthrin

Tryptanthrin (1a), whose structure has been established by X-ray crystallography,<sup>12</sup> and also studied in detail by e.g. NMR spectroscopy,<sup>10</sup> is a compound with a long history<sup>13</sup> and the compound was obtained by O'Neill as early as 1892. However, the old<sup>14–19</sup> synthetic methods, which include<sup>14</sup> treatment of isatin with hot aqueous KMnO<sub>4</sub>, are limited in scope and give poor yields. The method<sup>19</sup> of Zeide and Chelintsev, which involves nucleophilic substitution of 2-chlorobenzoic acid with 2-aminoquinoline followed by cyclization to 4, and subsequent oxidation of 4 with CrO<sub>3</sub> to 1a, is of special interest as it is relatively simple and should be considered for the synthesis of certain analogues. This method seems to have fallen into oblivion.<sup>16</sup>

For the synthesis of tryptanthrin itself and several analogues the most efficient technique is to heat isatin (5a) and isatoic anhydride (2H-3,1-benzoxazine-2,4-(1H)-dione) 6 in pyridine for ca 1 hr followed by cooling and collection of the crystals formed (75-95% yield). N-Methylpiperidine was found to be a useful catalyst in this condensation.

In order to get high yields it was found to be necessary to add an agent to trap the water formed in the condensation ( $5a + 6 \rightarrow 1a$ ). Diisopropyl carbodiimide or an excess of isatoic anhydride can be conveniently used<sup>20</sup> for this purpose. In this connection it was found that although isatoic anhydride can be conveniently recrystallized from dry pyridine it decomposes in aqueous pyridine.

## Other indolic constituents from the fruit of Couroupita guianensis Aubl.

The dried and powdered fruits were extracted with an alkaline  $Na_2S_2O_4$  solution. Aeration yielded (0.085%) indigo (7) which proved to be the major indolic constituent. However, it should be noted that the freshly cleaved fruits are colorless but do become dark-green within 60 sec. The structure of the indigo-generating compound has not been determined.

Chromatographic separation of an extract (CHCl<sub>3</sub>) from a dried and powdered fruit yielded except 1a also indirubin (8a), a common congener<sup>21,22</sup> of indigo, and a small amount of isatin (5a), which thus now for the first time has been isolated from plant material.<sup>23</sup> In this connection a recent report by Kapadia<sup>24</sup> concerning the isolation of the isatin derivative 5b from the plant *Melochia tomentosa* L. is of interest.

Indirubin was earlier isolated from Couroupita quianensis Aubl. by Sen et al.<sup>2</sup> but these workers failed to identify it and called the compound couropitine B, which thus is a superfluous name. The monoacetyl derivative (reported m.p. 186°) obtained by Sen seems to be the N-acetyl derivative **8b** although the melting point was found to be 214–215°. The N,N'-diacetylated derivative 8c has earlier been reported by de Diesbach and Wiederkehr<sup>25</sup> as a product from the interaction of indirubin with acetyl chloride in refluxing acetic anhydride. However repetition of this experiment only yielded **8b**.

Finally, recent reports<sup>26</sup> <sup>32</sup> by Chinese workers that indirubin inhibit Lewis lung carcinoma and Walker carcinosarcoma 256 is of considerable interest. The Nacetyl derivative **8b** showed equal or slightly higher activity. The corresponding N-ethyl derivative seems<sup>30</sup> to be even better.

### **EXPERIMENTAL**

IR spectra were obtained using a Perkin Elmer 257 instrument and mass spectra were recorded with an LKB 9000 mass spectrometer. UV spectra were recorded on a Perkin-Elmer Hitachi 200 spectrophotometer. The data of the following compounds were in complete agreement with those previously given in literature, 1a d. 8a.



7



2







**8x** R<sub>1</sub>=R<sub>2</sub>=H **8b** R<sub>1</sub>=H, R<sub>2</sub>=COCH<sub>3</sub> **8c** R<sub>1</sub>=R<sub>2</sub>=COCH<sub>3</sub>

Extraction of the fruits from Couroupita quianensis. Dried and powdered fruits (1 kg) of Couroupita quianensis were extracted with CHCl<sub>3</sub>. The evaporated extract was dissolved in CH<sub>3</sub>Cl<sub>2</sub>, filtered (to remove some indigo) and filtered through a column of silica gel to remove the rest of the indigo.

The resulting soln was concentrated and chromatographed on silica gel using  $CH_2Cl_2$  containing slowly increasing amounts of MeOH as eluent. The following compounds were isolated in order of elution (due to the colour of the compounds the separation is easy to follow): tryptanthrin (1a) 140 mg, indirubin (8a) 38 mg; isatin (5a) 8 mg. The indigo containing residues were sublimed yielding indigo (655 mg). This value might be compared with the amount (850 mg) obtained by extraction of the dried fruits (1 kg) with an alkaline soln of  $Na_2S_2O_4$ , followed by aeration, filtration, drying and sublimation.

Indirubin (8a).<sup>33</sup> 2-Chloroindolenin-3-one (1.65 g)<sup>34</sup> and 2indolinone (1.33 g) were refluxed in dry toluene (40 ml) for 1 hr, whereupon the solid formed was collected washed with toluene and recrystallized from dimethyl sulfoxide, yield 2.6 g (96%,a); m.p. > 340°.

Acetylation of indirubin (8a). Acetyl chloride (0.071 ml) was added to a soln of indirubin (262 mg) in dry pyridine (8.0 ml). The mixture was then beated (45", 2 hr) and quenched by addition of water. The 8b formed was extracted by CH<sub>2</sub>Cl<sub>2</sub>. After drying and concentration the residue was chromatoographed on a preparative TLC-plate using CH<sub>2</sub>Cl<sub>2</sub> containing MeOH (1°<sub>o</sub>) as eluent. Under these conditions 8a had the  $R_{f^{-1}}$  value 0.65 and **8b** the  $R_{f^*}$  value 0.83. The isolated yield of **8b** was 210 mg (70%): m.p. 214–215° (lit.<sup>2</sup> m.p. 186°); 1R (K.Br) 3310, 1710, 1680, 1620, 1600, 1460, 1370, 1300, 1270, 1140, 1050, 900, 780, 750 cm<sup>-1</sup>; MS, m/e 304 (M<sup>+</sup>, 45), 262 (100), 234 (55), 220 (18), 205 (23); UV/VIS  $\lambda_{max}$  (EtOH) 204 (log s 4.32), 230 (4.27), 290 (4.16), 360 (3.56), 549 (3.90) nm.

6,12-Dihydro-6,12-dioxoindolo[2,1-b]quinazoline: tryptanthrin (1a)

Method A. Isatin (7.35 g) and isatoic anhydride (8.15 g) was added to a well-stirred solution of N-methylpiperidine (0.2 ml) and disopropyl carbodiimide (5 ml) in dry pyridine (35 ml) at 60-65°. A clear soln was quickly obtained followed shortly by a brisk evolution of CO<sub>2</sub>. When the evolution subsided the temp was slowly (during 10 min) increased to 75°. During this operation crystallization of 1a usually commensed. To complete the reaction a final heating period (30 min) at 95-100° is suitable. Finally the mixture was cooled and the crystals collected, washed with MeOH and dried, yield 11.53 g (93°<sub>6</sub>) m.p. 267-268° (lit.<sup>10</sup> m.p. 265-266°). If the drying agent is omitted the yield drops to 68%.

Method B. 2-Chloroindolenin-3-one  $(8.33 \text{ g})^{34}$  and anthranilamide (6.80 g) were refluxed in CH<sub>3</sub>CN (60 ml) for 15 min, whereupon the solid formed was collected washed with water and recrystallized from AcOH or dimethyl sulfoxide, yield 10.17 g (82%) m.p. 267–268°.

The following compounds were similarly prepared (using method A): 1b yield  $(82^{\circ}_{o})$  m.p. 285-286°, 1c yield  $(76^{\circ}_{o})$  m.p. 303-305°, 1d yield  $(84^{\circ}_{o})$  m.p. 326-328°.

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