deshielding effect of the oxygen function at the 4-position [5]. The appearance of one aromatic proton at lower field (δ 7.5) indicates that the oxygen function is present at C-4 of compound **1**.

Most quinolone alkaloids of the Rutaceae contain an isopentyl group at position 3 and possess an oxygen function at position 8. It has been suggested [6] that a possible biosynthetic precursor of these alkaloids is **2**. From biogenetic consideration and spectral evidences the structure of ravesilone has been assigned as **1**. The isolation of ravesilone provides strong circumstantial evidence for the above idea.

EXPERIMENTAL

All mps are uncorr. UV and IR spectra were recorded in EtOH and as KBr pellets, respectively.

Isolation of **1**. Air dried finely powdered leaves (1 kg) of *R. spectabilis* (Farm) were first extracted with petrol (60–80°) for 36 hr. After this extraction, the residue was re-extracted with C_6H_6 for 36 hr. The solvent was then distilled off. The residue was taken up in Et_2O and fractionated into neutral, acidic and basic fractions in the usual way. The acidic fraction was then dissolved in a small vol of C_6H_6 and chromatographed over silica gel (400 g). The column was eluted with petrol, C_6H_6, C_6H_6–EtOAc (5:1) and EtOAc. From the C_6H_6–EtOAc eluate a solid was obtained. This was recrystallized from EtOH–petrol (40–60°) yielding a homogeneous white crystalline solid mp 272°. TLC (C_6H_6–$CHCl_3$; 1:1, R_f 0.68). (Found: C, 69.40; H, 5.98; N, 6.01. Calculated for $C_{15}H_{17}NO_3$; C, 69.46; H, 6.56; N, 5.41%.)

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INDOLIC COMPOUNDS IN THE LEAVES OF *TECOMA STANS*

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Key Word Index—*Tecoma stans*; Bignoniaceae; indole; skatole; tryptophan; tryptamine; anthranilic acid.

Abstract—Indole, tryptophan, tryptamine and skatole were isolated from the leaves of *Tecoma stans*. Anthranilic acid was also identified in its free form, in contrast to its glucoside, in *Jasminum grandiflorum*. The presence of both indole and anthranilic acid in the leaves of *Tecoma stans* indicates that they are the true substrate and product of indole oxygenase from the leaves of *Tecoma stans*.

INTRODUCTION

The indole ring is present in many physiologically important molecules and hence it has attracted the increasing attention of biochemists in recent years. The presence of indole itself was detected in jasmine oil as early as 1899 [1]. Indole was also found in the perfume of *Hevea brasiliensis* and *Raudia formosa* [2]. Later, Sack [3] reported the occurrence of indole in the wood of *Celtis reticulosa*. Sack [4] detected indole in citrus, coffee and mango plants, but no enzymes which metabolize indole from any of these plants except from jasmine were isolated [5]. A few reports are available on indole metabolizing enzymes from *Tecoma stans* [6, 7] and *Zea mays* [8], but nothing is known about the indolic compounds present in these plants. The enzymes from *T. stans* degrade indole to anthranil [6] and anthranilic acid [7]. Although indole was the best substrate tested, it may not be the true substrate. The aim of the present investigation was to carry out such a systematic study.

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RESULTS AND DISCUSSION

Various indolic compounds such as indole, tryptophan, tryptamine and skatole were isolated from the leaves of *Tecoma stans L.* The R_f values of these compounds and

Table 1. Identification of indolic compounds in the leaves of *Tecoma stans*

	R_f value in solvent system			Colour reaction with Ehrlich reagent
	A	B	C	
Tryptophan	0.00	0.26	0.12	Blue
Tryptamine	0.25	0.41	024	Blue
Indole	0.77	0.83	0.87	Violet
Skatole	0.75	0.81	0.91	Blue

Solvent system: A = chloroform–96% acetic acid (19:1); B = isopropanol–ammonia–water (10:1:2); C = chloroform–methanol–acetic acid (15:4:1).

Table 2. Identification of anthranilic acid in *Tecoma* leaves

Property	
R_f value in	
Ethanol–ammonia–water (18:1:1)	0.52
Isopropanol–ammonia–water (20:1:2)	0.39
Butanol–acetic acid–water (4:1:1)	0.88
Colour reaction with Ehrlich reagent	Yellow
λ_{max} in ethanol	221 nm
	247 nm
	335 nm

their colour reactions with Ehrlich reagent are shown in Table 1. Indole might be formed from tryptophan by the well-known tryptophanase reaction [9, 10], but no such enzyme has been reported in *Tecoma* leaves. Indole thus formed is converted to anthranil by indole oxidase [6]. This system is a cuproflavoprotein and is atabrine-sensitive. An atabrine-insensitive enzyme system, indole oxygenase, converts indole to antharanilic acid [7]. The presence of indole in the leaves of *T. stans* shows that it is the true substrate of both indole oxidase [6] and indole oxygenase [7].

In addition to various indolic compounds, the presence of anthranilic acid was also detected in *Tecoma* leaves. The R_f values, spectral properties and colour reaction with Ehrlich reagent of the authentic and isolated anthranilic acids are given in Table 2. This compound also occurs in *Jasminum grandiflorum* as the glucoside (unpublished results), where anthranilic acid formed by the action of indole oxygenase [5] accumulates as anthranilic acid glucoside in leaves and flower buds, and is converted to methylanthranilate.

An enzyme system which catalyses the conversion of anthranilic acid to catechol was purified from a cell-free leaf extract of *T. stans* [11–13]. This reaction proceeds via 3-hydroxyanthranilic acid and *o*-aminophenol to catechol. Since anthranilic acid is not accumulated as its glucoside in *T. stans*, free anthranilic acid could be detected. In *Jasminum grandiflorum*, anthranilic acid does not undergo any further metabolism except its conversion to methylanthranilate, while a pathway exists in *T. stans* whereby this compound is converted to catechol. The presence of both indole and anthranilic acid in the leaves of *T. stans* indicates that they are the true substrate and the true product of indole oxygenase in this plant [7], and this provides additional support for the presence of indole oxygenase in the leaves of *T. stans* [7].

EXPERIMENTAL

Extraction of indolic compounds from plant material. Leaves of *T. stans* (200 g) were homogenized with 80% MeOH in the ratio 1:1 and the resultant slurry was refluxed for 1 hr. The extract was filtered, concd to 10 ml in a flash evaporator, and divided into two.

Detection of indolic compounds. The first part was used to separate various indolic compounds by TLC [14] using different solvent systems (Table 1). Indolic compounds were located by UV and detected by spraying the plate with Ehrlich reagent [15].

Detection of anthranilic acid. The second part was subjected to PC on Whatman No. 1 paper using different solvent systems (Table 2). Anthranilic acid was located on the chromatograms by its fluorescence and also by its colour with Ehrlich reagent [15]. It was eluted with EtOH and the spectral properties were compared with those of authentic anthranilic acid.

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