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Note

δ-Aminolaevulinic acid synthetase

Synthesis of δ-aminolaevulinic acid pyrroles and their separation by thin-layer chromatography

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δ-Aminolaevulinic acid (ALA) synthetase is the rate-limiting enzyme of porphyrin biosynthesis in animal, bacterial and plant cells[1–10]. It catalyses the synthesis of δ-aminolaevulinic acid by the condensation of glycine and succinyl coenzyme A[1,11]. The activity of this enzyme is increased in the livers of patients with acute intermittent porphyria[12], a genetic abnormality of the regulation of heme synthesis, and in the livers of animals rendered porphyric by means of variety of drugs, referred to as experimental porphyria[13].

Granick and Urata[14] induced an experimental porphyria in guinea pigs by the administration of 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC) and showed that there is a large increase in porphyrin synthesis resulting from a greater increase in the ALA-synthetase activity in liver mitochondria. Later, working with the elegant chick embryo liver cells technique, Granick[15] showed that the increased activity of this enzyme, induced by DDC, results from an increased synthesis of this enzyme, rather than from activation of the inactive enzyme. ALA-synthetase activity is usually measured by converting the ALA produced by the enzyme during incubation into a pyrrole by condensation with either ethyl acetoacetate or acetylacetone[16]. The pyrrole formed is reacted with Ehrlich's reagent and measured. This interest in ALA-synthetase has prompted us to study ALA pyrroles. The purpose of this paper is to describe the synthesis of ALA pyrroles from δ-ALA acid hydrochloride and β-keto esters and the use of TLC to characterize these compounds. The separation by TLC of ALA pyrroles does not seem to have been studied. This study has given important information regarding the effect of various ester and alkyl substituents in the 3- and 2-positions on the mobility of the ALA pyrroles during TLC separation.

MATERIALS AND METHODS

Benzyl acetoacetate was obtained by the method described in the literature[17]. Methyl, ethyl and *tert.*-butyl acetoacetate and ethyl propionyl and ethyl butyryl acetate were purchased from Aldrich (Milwaukee, Wisc., U.S.A.). δ-Aminolaevulinic acid hydrochloride was synthetized according to the method of Shemin *et al.*[5]. Ultraviolet absorption spectra were determined in absolute ethanol in a Unicam SP-800

TABLE I

UV AND IR DATA FOR ALA PYRROLES

Compound	R_1	R_2	Yield (%)	M.p. (°C)	Infrared spectrum (cm^{-1})		Ultraviolet spectrum				Elemental analysis (%)					
					NH	CO	λ_{max} (nm)	$\varepsilon \times 10^{-3}$	λ_{max} (nm)	$\varepsilon \times 10^{-3}$	C		H		N	
											Calculated	Found	Calculated	Found	Calculated	Found
1a	CH_3	CH_3	61	178–180	3300	1710 1665	232	8.37	260	5.8	56.86	56.62	6.20	6.15	6.63	6.50
2a	CH_3	C_2H_5	50	164–166	3300	1710 1665	233	8.25	260	5.79	58.65	58.55	6.71	6.56	6.22	6.17
3a	CH_3	$CH_2C_6H_5$	50	143–145	3280	1705 1665	233	11.8	260	7.42	66.88	66.65	5.96	5.80	4.88	4.92
4a	CH_3	$C(CH_3)_3$	67	144–146	3320	1705 1665	232	8.97	260	6.02	61.64	61.57	7.56	7.63	5.53	5.44
5a	C_2H_5	C_2H_5	45	153–155	3300	1710 1660	233	13.01	263	9.7	60.24	60.26	7.16	7.23	5.85	5.74
6a	C_3H_7	C_2H_5	37	123–125	3300	1715 1660	233	8.52	263	6.1	61.64	61.49	7.56	7.47	5.53	5.50

spectrophotometer. Infrared spectra were obtained in Nujol on a Perkin-Elmer Model 137E infrared spectrometer. All melting points are uncorrected.

Standard conditions for the synthesis of ALA pyrroles

A solution of β-diketone (0.01 mole) in 20 ml of phosphate buffer (0.25 *M*; pH 6.6) was treated with equimolar amount of δ-aminolaevulinic acid hydrochloride (0.01 mole) and refluxed for 15 min. The solution was cooled and pH was adjusted to 7. The pyrrole formed was separated by filtration, washed and crystallized from aqueous ethanol.

Thin-layer chromatography (TLC) was carried out according to Roomi[18,19]. A suspension of 30 g of silica gel G (E. Merck, Darmstadt, G.F.R.) in 60 ml of water was spread on glass plates (20 × 20 cm) to a thickness of 250 μm with a Desaga applicator. The plates were dried at 105–110° for 30 min and stored in a desiccator. A 0.1% solution of the compounds in methanol was prepared and 2 μg of each compound was spotted 2 cm from the edge of the plate. The plate was then developed with 150 ml of the solvent system diethyl ether–*n*-hexane containing 2% glacial acetic acid (1:1). Usually 45 min were required for the solvent to travel a distance of 12–15 cm. The plates were then dried, sprayed with Ehrlich reagent[20] and, after keeping for 5 min at 100°, the pyrroles appeared as blue spots.

RESULTS AND DISCUSSION

Fig. 1 lists the various β-diketo esters used and ALA pyrroles formed and their separation by TLC. The UV and IR spectra of these compounds are recorded in Table I. The IR spectra of all the compounds showed bands around 3300 cm^{-1} due to the N–H and at 1710 and 1660 cm^{-1} due to C=O. The UV spectra of these compounds showed a maximum around 233 nm and a minimum around 260 nm. Reaction of these pyrroles with modified Ehrlich's reagent[16] gave a pink solution, which showed a maximum at 552 nm and a shoulder at 525 nm. Fig. 2 shows the absorption spectra of ALA pyrrole obtained with *tert.*-butoxy ester, which has

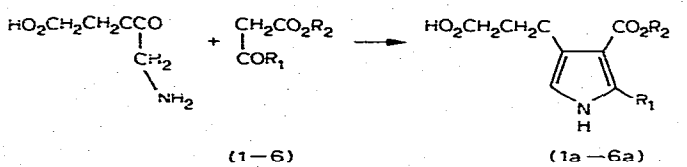

Fig. 1. Reaction scheme for synthesis of ALA pyrroles and their separation by TLC. Solvent system used: diethyl ether–*n*-hexane containing 2% glacial acetic acid (1:1).

No.	*Pyrrole*		*R_F × 100*
1a	$R_1 = CH_3$	$R_2 = CH_3$	24
2a	$R_1 = CH_3$	$R_2 = C_2H_5$	29
3a	$R_1 = CH_3$	$R_2 = CH_2C_6H_5$	33
4a	$R_1 = CH_3$	$R_2 = C(CH_3)_3$	43
5a	$R_1 = C_2H_5$	$R_2 = C_2H_5$	38
6a	$R_1 = C_3H_7$	$R_2 = C_2H_5$	43

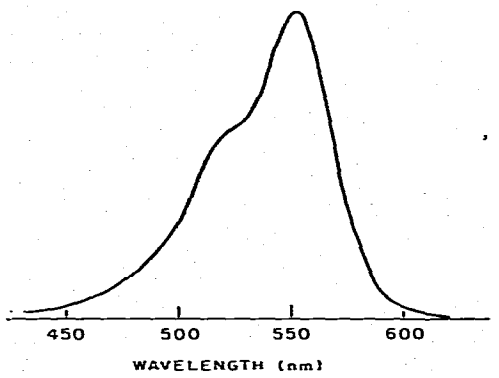

Fig. 2. Absorption curve for *tert.*-butoxy ALA pyrrole.

absorption maximum at 552 nm. The mechanism and the steps involved in the colour reaction of ALA pyrroles with Ehrlich's reagent are shown in Fig. 3.

The separation of ALA pyrroles (1a–6a) by TLC is shown in Fig. 1. 2-Methyl-3-carbethoxy-4-propionic acid pyrrole (2a) has higher R_F value than the 2-methyl-3-carbomethoxy-4-propionic acid pyrrole (1a) and are readily separated. Replacement of 2-methyl in 2a by either ethyl (5a) or propyl (6a) makes it less polar and it therefore then moves faster.

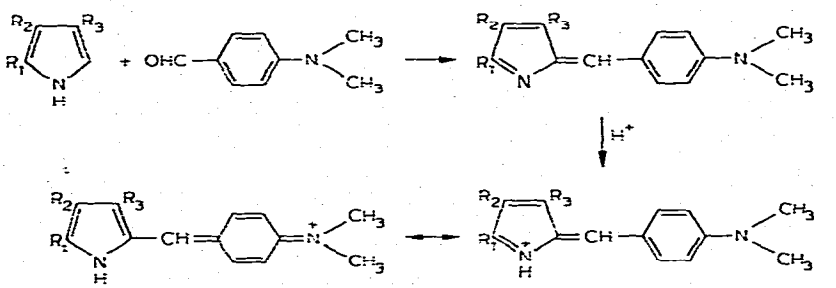

Fig. 3. Mechanism of colour reaction of ALA pyrroles with Ehrlich's reagent.

Similar substitution of 3-carbethoxy in 2a by more bulky groups, *viz.*, *tert.*-butoxy (4a) and benzyl (3a), also decreases the polarity of the compound and results in an increase R_F value. Hence the ALA pyrroles with different esters groups (methyl (1a), ethyl (2a), benzyl (3a) and *tert.*-butoxy (4a)) in the 3-position are separated from each other. Similar resolution was achieved among the ALA pyrroles having different alkyl substituents (methyl (2a), ethyl (5a) and propyl (6a)) in the 2-position. Thus all the ALA pyrroles synthesized in the present investigation can be separated from each other with the exception of 4a and 6a, which have the same R_F value.

Recently, ALA-synthetase activity has been measured in tissue obtained from liver biopsies[21] and in cultured cells[22,23]. The small amounts of tissue obtained by these procedures generate a small amount of ALA. The assay procedure utilizes ethyl acetoacetate to form the pyrrole prior to addition of Ehrlich's reagent. As *tert.*-buty

acetoacetate ester gives a better yield than ethyl acetoacetate when used to form the ALA pyrrole, this compound may be more desirable to use in assays of ALA-synthetase.

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