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UV SPECTRA OF INDOLES IN STRONG SULPHURIC ACID

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Abstract—Measurement of the UV spectra in 12·7 M H_2SO_4 at 30° and 70° provides a simple, quantitative and specific method for the identification of variously substituted, naturally occurring plant indoles in μg quantities.

INTRODUCTION

IN THE course of a study on the interaction of indoleacetic acid (IAA) with gibberellic acid in germination of barley seeds, the conjugate of IAA and *L*-aspartic acid (L-Asp) was isolated and the structure was confirmed by synthesis.[1] The involatile nature of IAA-L-Asp limited the usefulness of MS analysis and led us to investigate, in more detail, the use of UV as a supplementary means for identification of 3-substituted indoles. In methanol many 3-substituted indoles have essentially identical UV spectra. However, in strong sulphuric acid the spectral behaviour of chemically very similar 3-substituted and other indoles is different.

As the amounts of natural indoles from plant sources are often small, only measurements requiring microgram quantities of material are useful. The MS of a number of 3-substituted indoles have been published;[2] and techniques have been developed for the measurement of IR spectra on a small scale.[3,4] More recently fluorescence spectra measured *in situ* on TLC plates, after acid spray, have been found to differentiate between substituted indoles;[5] and electron donor–acceptor complexes have been studied for use in qualitative and quantitative identification.[6]

Hinman and Lang[7] in a study of the acidity functions of various indoles reported the UV spectra of a number in 12 M H_2SO_4. Where the spectra changed with time, they were extrapolated back to zero time; however, the changes were not recorded.

RESULTS AND DISCUSSION

The changes with time and temperature of a number of 3-substituted indoles in strong sulphuric acid were examined and it was observed that the behaviour of the indoles (compounds I–XVIII) at either 30° or 70° serves to distinguish between them. Using a scale

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TABLE 1. UV SPECTRA OF 3-SUBSTITUTED INDOLES

No.	Compound	MeOH Conc. ($M \times 10^5$)	MeOH λ_{max} nm ($\epsilon \times 10^{-2}$)	H_2SO_4 Conc. ($M \times 10^5$)	Time (min)	H_2SO_4 λ_{max} nm ($\epsilon \times 10^{-2}$)
I	Indole-3-pyruvic acid	6·5	323(216); 234(240)	11	10	340(106); 258(25); 225(54)
					20	340(75); 260(29); 225(54)
					30	340(47); 288sh(39); 260(31); 225sh(54)
					60	340sh(27); 288(42); 260(38); 225sh(61)
II	Indole-3β-acrylic acid	6·8	323(216); 274(120); 226(206)	11	10	356(38); 260(129); 253(127)
					1320	350(29); 272sh(55); 260(89); 253(92)
III	Indole-3-glyoxyl-amide	7·3	323(116); 272(99); 267(107); 255(123)	11	30	376(91); 271(141); 249(112)
					1320	374(80); 271(122); 251(93)
IV	Indole-3-glyoxylic acid	7·0	310(94); 273sh(87); 266(103); 254(106)	12	‡	390(103); 275(138); 250(103); 243sh(97)
V	Indole-3-aldehyde	9·7	296(137); 260(124); 243(140)	8·2	‡	338(129); 267(254); 261(212); 241sh(165); 235(181)
VI	Indole-3-acetalde-hyde (Bisulphite compound)	—	—	28	2	288(45); 241(41); 235(44)
					10	350sh(19); 293(47); 264sh(29); 241sh(37); 234(45)
					30	345sh(30); 292(41); 264(35); 230(54)
					60	337sh(33); 290(39); 262(40); 227(63)
VII	Indole-3-acetyl aspartic acid	6·6	289(52); 280(64); 272(61); 221(250)	43	2	290(34); 241(32); 235(34)
					10	287(13); 267(12); 260(11); 241(14); 226sh(20)
					180	284(16); 267(16); 260(15); 241(15); 226sh(36)
					420	281(21); 267(22); 260(21); 241sh(19); 226(51)
VIII	Indole-3-acetone	9·7	289(59); 281(71); 274(68); 225(182)	28	2	348sh(13); 288(53); 241(47); 235(51)
					10	348(33); 292(54); 241(47); 235(51) Little further change over 24 hr
IX	Indole-3-butyric acid	13	291(55); 282(66); 276sh(61); 229(152)	29	10	354(2); 290(52); 241(46); 236(48)
					60	354(17); 290(50); 241(47); 236(49)
					120	354(37); 290(48); 242(50); 237(50)
					180	354(53); 290(46); 242(52); 237(52)
X	Tryptophan	13	289(55); 280(65); 274sh(63); 227(148)	25	10	290(50); 241(46); 236(49)
					240	288(50); 241sh(48); 231(74)
					600	287(51); 234(92)
					1380	285(53); 236(97)
XI	Indole-3-acetamide	9·1	290(59); 281(70); 274(66); 225(198)	24	10	291(52); 241(46); 236(48)
					180	295(58); 262sh(30); 241sh(37); 227(69)
					600	295(49); 269sh(38); 261(32); 241sh(29); 227(87)
					1380	291(32); 266(30); 260(32); 227(92)
XII	Indole-3-aceto-nitrile	9·0	288(56); 278(68); 272(68); 224(189)	28	10	295(50); 242(46); 237(49)
					180	293(50); 262sh(26); 241(42); 231(54)
					600	294(49); 267sh(31); 262sh(29); 241sh(33); 227(74)
					1380	291(36); 268(29); 260(29); 229(81)
XIII	Tryptamine HCl	9·0	290(56); 281(68); 274(65); 225(187)	26	10†	291(45); 241(39); 236(41)
					1080	291(44); 241sh(41); 234(53)
					30	288(42); 234(86)
					60	287(41); 235(92)
XIV	Tryptophol	10	291(58); 282(70); 275sh(64)	29	10*	289(46); 240sh(43); 235(47)
					60	288(42); 264sh(32); 240sh(45); 235 (48)
					120	287(38); 263(35); 258sh(30); 240sh(47); 235(49)
					180	287(35); 263(37); 257sh(33); 240sh(47); 235(49)
XV	Indole-3-acetic acid	6·5	289(52); 280(63); 273(60); 223(250)	21	10*	289(50); 240sh(40); 234(47)
					60	287(48); 240sh(53); 232(70)
					120	286(46); 264(38); 232(90)
					180	285(43); 264(43); 232(99)
XVI	Indole-3-lactic acid	9·4	290(55); 282(65); 275(62); 227(188)	30	10*	287(40); 240sh(43); 234(47)
					60	285(36); 262sh(23); 240sh(44); 234(50)
					120	284(31); 264sh(27); 240sh(45); 233(51)
					180	280(28); 265sh(28); 240sh(45); 233sh(51)

TABLE 1—*continued*

No.	Compound	MeOH Conc. ($M \times 10^5$)	MeOH λ_{max} nm ($\epsilon \times 10^{-2}$)	H_2SO_4 Conc. ($M \times 10^5$)	H_2SO_4 Time (min)	H_2SO_4 λ_{max} nm ($\epsilon \times 10^{-2}$)
XVII	Indole-3-propionic acid	9·3	291(58); 282(68); 275sh(63); 227(206)	22	10*	288(52); 240(49); 235(53)
					180	328sh(19); 288(49); 240sh(67); 236(71)
					360	328sh(25); 285(45); 240sh(79); 237(81)
					600	325(33); 279sh(42); 238(87)
XVIII	Skatole	9·4	291(55); 282(66); 275sh(62); 228(204)	24	10*	287(50); 240(43); 235(46)
					300	285(47); 240(45); 235(47)
					600	283(45); 240(47); 235(49)

* Spectra run at 70°; the 10 min run refers to a solution which has been left for 12 hr at 30°, then equilibrated at 70° for 10 min.

† The first two spectra (10 and 1080 min) are at 30°. The others (30 and 60 min) are at 70°.

‡ Unchanged with time.

expansion unit with the spectrophotometer, satisfactory results were obtained with 0·5–5 μg quantities.

TABLE 2. UV SPECTRA OF INDOLES

No.	Compound	MeOH Conc. ($M \times 10^5$)	MeOH λ_{max} nm ($\epsilon \times 10^{-2}$)	H_2SO_4 Conc. ($M \times 10^5$)	H_2SO_4 Time (min)	H_2SO_4 λ_{max} nm ($\epsilon \times 10^{-2}$)
XIX	Indole	8·4	288(55); 281sh(67) 278(67); 272(69); 222(191)	28	10	359(15); 282(35); 261(57); 255sh(54); 241(49); 235(47)
					60	359(11); 283(39); 261(50); 255sh(45); 241(44); 235(44)
					120	359(8); 283(42); 261(44); 255sh(38); 241(42); 235(42)
					1440	283(54); 240(36); 235(39)
XX	Indole-2-carboxylic acid	7·5	291(185); 223(199)	12	20	318(123); 235(95)
					60	313(138); 237(122)
					180	310(142); 239(143)
					1560	307(145); 246sh(165); 242(178)
XXI	5-Hydroxy tryptophan	9·8	312sh(37); 300(47); 276(61); 225(161)	28	10	320(50); 249(54)
					360	319(49); 289sh(35); 249(53)
					1440	317(49); 289(41); 246sh(51); 232(63)
XXII	Indoxyl-β-D-glucoside	16	289(42); 282(46); 272sh(40); 230(119)	17	10	396(20); 285sh(18); 250(96)
					240	394(29); 285sh(22); 250(96)
					1260	390(33); 285sh(28); 250(96)
XXIII	Indole-5-carboxylic acid	4·7	275(64); 236(400)	14	10	273(94); 235sh(53); 227(60)
					360	274(94); 235sh(59); 226(64)
					1380	274(96); 235sh(72); 225(77)
XXIV	Indoxyl-1,3-diacetate	8·1	300(91); 291(85); 271sh(90); 262(100); 237(198)	5·6	‡	317(50); 264(175); 243sh(274); 238(286)
XXV	5-Hydroxyindole-3-acetic acid	10	312sh(40); 299(52); 277(72); 227(166)	23	10*	318(51); 248(51)
					180	315(50); 248(52)
					360	313(49); 248(53)
XXVI	5-Hydroxy-indole	10	310sh(34); 298(46); 272(77); 223(148)	26	10†	308(50); 280sh(39); 245(52)
					10	310(51); 246(42)
					360	309(51); 245(43)
XXVII	Indole-1-propionic acid	10	292(52); 285sh(64); 281(68); 273(68); 227(176)	25	10*	284(48); 241(30); 236(31)
					360	282(47); 241(32); 235(33)

* Spectra run at 70°; the 10 min run refers to a solution which has been left for 12 hr at 30°, then equilibrated at 70° for 10 min.

† The first spectrum is read after 10 min at 30°. The 10-min spectrum refers to a solution which has been left for 12 hr at 30°, then equilibrated at 70° for 10 min; the 360 min spectrum has been left a further 350 min at 70°.

‡ Unchanged with time.

The eighteen 3-substituted indoles may conveniently be divided into three classes: (i) Compounds (I)–(V): initial spectra in H_2SO_4 differ significantly from that of IAA.

(ii) Compounds (VI)–(XII): initial spectra in H_2SO_4 are similar to that of IAA but change significantly with time at 30°. (iii) Compounds (XIII)–(XVIII): initial spectra in H_2SO_4 are similar to IAA, change little at 30°, but can be distinguished by changes at 70°.

The details of the spectra are tabulated (see Table 1). The spectrum in methanol of each indole is included (except for indole-3-acetaldehyde (VI) which was used as its bisulphite complex). Spectra in H_2SO_4 were measured at 70° if little or no change occurred after 12 hr at 30°.

The likely possibility of cyclisation to position 2 in some of the 3-substituted indoles, is demonstrated clearly in the spectra of indole-3-butyric acid (IX) in H_2SO_4. The product was isolated and shown to be 1-ketotetrahydrocarbazole by comparison with an authentic specimen.[8]

The UV spectra of nine other indoles are tabulated in Table 2. The distinctive differences in spectra of these compounds suggest that the method has wider application.

EXPERIMENTAL

The reagent (H_2SO_4) is prepared by adding commercial conc. H_2SO_4 to half its own vol. of dist. H_2O. Titration shows that the molarity of the solution is about 12·7. Satisfactory results were obtained with five commercial preparations: BDH Aristar; BDH AnalaR; BDH Laboratory Reagent; Merck Suprapur and Merck GR. The spectra of each 3-substituted indole were measured in all 5 acids. The spectra tabulated in Tables 1 and 2 were measured in BDH AnalaR acid.

IAA-L-Asp was synthesized.[1] Tryptamine HCl was supplied by BDH and the remaining indoles by Sigma Chemical Company. Measurements were carried out in a 1 cm quartz cell on a Unicam SP 800 B spectrophotometer.

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