INDOLYL-3-PYRUVIC ACID OXIME AS THE PRECURSOR OF INDOLYL-3-ACETONITRILE[1]

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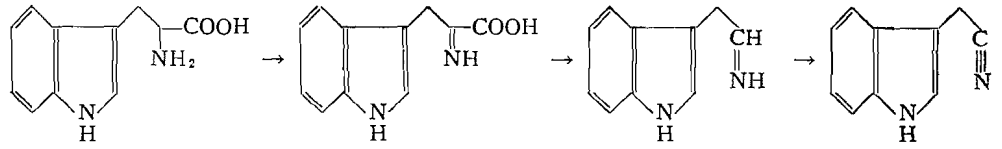
ABSTRACT

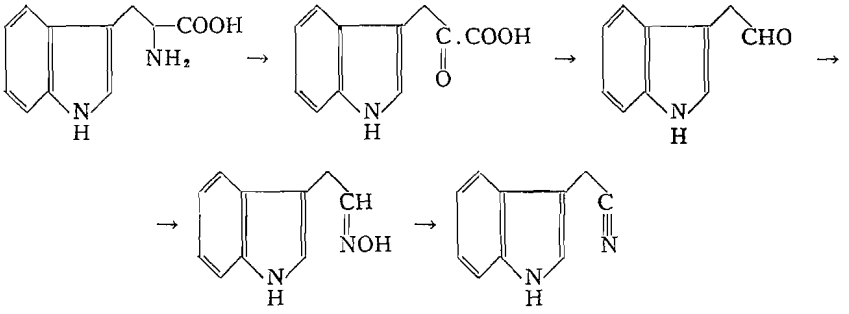
Chemical and chromatographic evidence is presented compatible with the hypothesis that the auxin indolyl-3-acetonitrile is derived in plants from indolyl-3-pyruvic acid oxime (anti HO– –COOH) by a concerted reaction.

Since the discovery that indolyl-3-acetonitrile (IAN) occurs naturally (1) and shows auxin activity in certain plant species, two hypotheses of its biosynthetic origin have been proposed. Both of these postulate tryptophan as the parent substance, but differ in the intermediate steps whereby the alanyl side chain of the amino acid is modified to $-CH_2-CN$ in the nitrile.

Sequence A was the first to be put forward (1).

(A) 

Experimental work (2) has established that IAN is indeed derived enzymatically from tryptophan in watermelon slices and in *Avena* coleoptiles. Incubation of the tissues with α-C^{14}-tryptophan, followed by paper chromatographic analysis of extracts, gave rise to a number of radioactive spots, one of which was assigned to IAN. Another highly active spot was tentatively ascribed to indolyl-3-pyruvic acid, whose occurrence in plant extracts (3) had been the subject of controversy (4, 5, 6). A modified sequence of steps (sequence B) for IAN biosynthesis was suggested as a result of this tracer work (2).

(B) 

Neither of the two hypotheses is entirely satisfactory. The first step of sequence A has many biochemical analogies, but amino acid oxidases, the type of enzyme required to catalyze such a reaction, are comparatively rare in plant tissues (7). The final step, dehydrogenation of an aldehyde imine to a nitrile, is without known chemical or biochemical precedent, and even the second step is unusual since in biochemical systems

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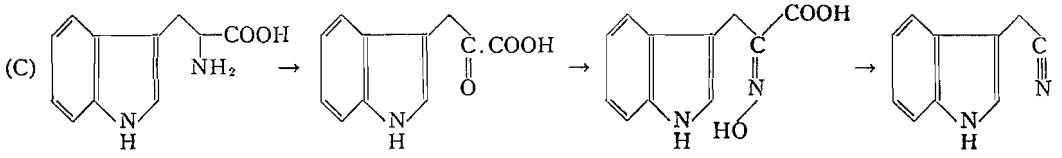
α-imino acids undergo rapid spontaneous hydrolysis to α-keto acids (8) rather than decarboxylation. The latter reaction is feasible, however, since in vitro primary amines catalyze α-keto acid decarboxylation (e.g. ref. 9) with the intermediate formation of N-substituted α-imino acids (Schiff's bases), which themselves readily decarboxylate (e.g. ref. 9). Such a mechanism, however, is no longer accepted for the enzymatic decarboxylation of α-keto acids (10).

Indirect evidence supporting the first step of sequence B, conversion of tryptophan to indolyl-3-pyruvic acid by transamination, has been accumulating (e.g. ref. 11). The only intermediate in sequence B which has not yet been found to occur is indolyl-3-acetaldoxime. Other oximes, and particularly α-keto acid oximes, have, however, been isolated from natural sources (e.g. ref. 12) and the formation of indolyl-3-acetaldoxime from indolyl-3-acetaldehyde and hydroxylamine is a tenable supposition, since indirect evidence for the occurrence of both these substances in plants is available (6 and 13 respectively). The fact that IAN biosynthesis from tryptophan is inhibited by dimedone, which is regarded as a specific aldehyde trapping agent, has been adduced as an important argument in favor of the implication of indolyl-3-acetaldehyde in the biogenetic sequence (14). The final step of sequence B, although easily accomplished chemically under vigorous, usually anhydrous, reaction conditions, is again without known biochemical analogy.

Neither sequence, however, accounts for the detection by Housley and Bentley (4, 15) of an acidic precursor of IAN in the water-soluble, ether-insoluble fraction of an extract of cabbage leaves. This precursor gave IAN when heated in aqueous solution pH 5.6 at 98–100° for 25 minutes. The same chemical reaction took place to some extent even at room temperature under basic, but particularly under acidic, conditions. Other data (16) also point to the occurrence of a water-soluble, ether-insoluble acidic precursor of IAN.

It should be noted that Housley and Bentley (15) do not present direct evidence bearing on the conversion of their precursor to the nitrile in vivo, but infer biological conversion on the basis of the precursor's growth-promoting properties and by analogy with its chemical behavior. Accepting this inference as a working hypothesis, it follows that any substance postulated as direct biological precursor of the nitrile should also be convertible to nitrile under mild chemical conditions. The identity of the precursor with either indolyl-3-acetaldehyde imine (sequence A) or indolyl-3-acetaldoxime (sequence B) must therefore be ruled out.

We have examined the chemical and chromatographic properties of synthetic indolyl-3-pyruvic acid oxime and have found them to be very similar to those of the IAN precursor described by Housley and Bentley. We wish to advance a new hypothesis for the biosynthesis of IAN (sequence C) with indolyl-3-pyruvic acid oxime as the key intermediate:



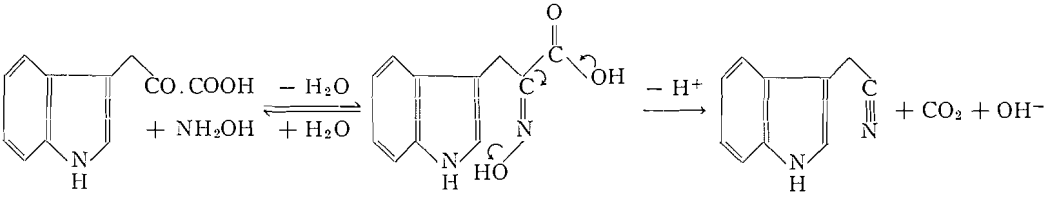
The first step in our sequence is identical with that of sequence B. The second step, in vivo conversion of α-keto acid to the corresponding oxime, is not a novel idea (13). Although indolyl-3-pyruvic acid oxime has not been isolated from natural sources, other

α-oximino acids have been obtained from plants (17) and from micro-organisms (e.g. ref. 12) and it has been shown that compounds of this type may be utilized biologically (18, 19), whereas oximes of carbonyl compounds other than α-keto acids are relatively inactive (18, 20). A transoximase system, catalyzing the transfer of =N–OH between α-keto acids, has been detected in a variety of tissues (21). α-Keto acid oximes are thus known as biochemical intermediates.

The novel feature of our sequence lies in the postulated concerted conversion of indolyl-3-pyruvic acid oxime to IAN. We have found that this reaction takes place readily in aqueous solutions pH 1 to 7 at moderate temperatures, as required for an IAN precursor by the results of Housley and Bentley.

Not only indolyl-3-pyruvic acid oxime, but all other α-keto acid oximes we have tested, yield the lower nitrile and CO_2 under these reaction conditions. The generality of this phenomenon was first recorded by Bouveault and Locquin (22) but has since been almost entirely overlooked, except in isolated instances (23). Considering the ease with which the conversion takes place it is not surprising that reaction of α-keto acids with hydroxylamine under the usual conditions gives oxime in poor yield only and that occasionally (e.g., indolyl-3-glyoxylic acid (24)) no oxime, but only nitrile is obtained. It is probable that Bauguess and Berg (25) obtained a very impure sample of indolyl-3-pyruvic acid oxime (m.p. indefinite, >175°) by this method. The compound (m.p. 154° (decomp.)) was obtained in 59% yield (26) by nitrosation of ethyl α-acetyl-β-(3-indolyl)-propionate.

When heated in 0.05 *M* H_2SO_4 for 3 hours, indolyl-3-pyruvic acid oxime is converted to IAN in 95% yield. The same product (60% yield) is obtained directly when equimolar amounts of indolyl-3-pyruvic acid and hydroxylamine hydrochloride are refluxed in aqueous solution, pH 1.



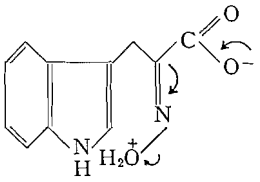
In further confirmation of the ease with which IAN formation takes place, decomposition of oxime to nitrile was found to occur readily even at low temperature. Table I

TABLE I

Yield of IAN on incubation at 41.5° for 24 hours of 20 mg indolyl-3-pyruvic acid oxime in buffer solutions, pH 1–7

Buffer	Initial pH	Final pH	IAN obtained	
			mg	% yield
HCl/KCl	1.0	1.0	6.44	45.0
HCl/KCl	1.6	1.6	7.36	51.4
HCl/KCl	2.0	2.0	7.51	52.5
Phthalic acid/hydrogen phthalate	3.1	3.0	6.73	47.0
Hydrogen phthalate/phthalate	4.0	3.6	5.41	37.8
Hydrogen phthalate/phthalate	5.0	4.5	1.85	12.9
$H_2PO_4^-/HPO_4^=$	7.0	6.3	0.78	5.5

shows the yields of nitrile at various pH values, when a dilute solution of oxime was incubated at 41.5° for 24 hours. The yield of product was optimal at pH 2, decreased at lower pH values, and dropped with rise in pH, but even at pH 7 nitrile was obtained. Although the decreased yields at pH <2 may be due entirely to side reactions leading to destruction of the indole nucleus, this is not likely in view of the good yield of IAN at pH 1 at 100° but may be a consequence of the existence at pH 2 of maximal amounts of the zwitterionic species, which would be the most favorable for a concerted elimination



of carbonic anhydride and water from the oxime. At pH <2 the carboxylate group (pK_a 3.4) would be more than 96% protonated, whereas at pH >2 the oxime OH is unlikely to bear a proton.

A concerted elimination of this type requires an anti (HO– –COOH) stereochemistry of the oxime. Only in very few instances (phenylglyoxylic acid (27), oxaloacetic acid (28), and possibly phenylpyruvic acid (29), glyoxylic acid (30), and α-ketoglutaric acid (19)) have syn- and anti-isomers of α-keto acid oximes been described. The stereochemistry of these oximes has not been reinvestigated by modern methods, and therefore remains uncertain, but on the basis of their classical investigations both Hantzsch (27, 28) and Meisenheimer (31) concur that, where only one isomer is known, the favored stereochemical arrangement of α-keto acid oximes is anti (HO– –COOH), as now required to account for the facility of their conversion to nitriles in aqueous solution by a concerted acid catalyzed mechanism.

Having established that indolyl-3-pyruvic acid oxime yields IAN under mild chemical conditions, as demanded for a precursor according to Housley and Bentley, it remained to compare the behavior on extraction and chromatography of Housley and Bentley's precursor with that of the oxime. In Table II the chromatographic behavior of indolyl-3-

TABLE II

	Precursor	Oxime	IAN
(1) R_f ranges			
2-PrOH/0.15 M NH_3 (4:1)	0.39–0.48	0.40–0.45	0.85–0.90
1-BuOH, saturated with 1.5 M NH_3	0.10–0.15	0.13–0.21	0.85–0.90
(2) Chromogenic sprays			
$FeCl_3/HClO_4$	Yellow	Mauve changing to greenish yellow	Violet blue changing to brown
HNO_2/HNO_3	Yellow	Yellow	Blue-purple changing to greenish yellow
p-Dimethylaminobenzaldehyde		Mauve changing to greenish yellow	Mauve changing to brown
(3) Fluorescence	Bluish-purple	Blue	Blue

pyruvic acid oxime is compared with that of Housley and Bentley's IAN precursor. The similarity of the R_f ranges of the two substances in the solvent systems used by Housley and Bentley is evident. Differences in color with Ehrlich's reagent and $FeCl_3$/perchlorate

may be accounted for by concentration effects. At low concentrations the mauve color given by indolyl-3-pyruvic acid oxime with these two reagents was exceedingly transient.

Complete analogy was found in the behavior of synthetic oxime and IAN precursor on partition between solvents: using a solution of indolyl-3-pyruvic acid oxime (20 mg in 15 ml 95% ethanol, adjusted to pH 3.2 with dilute sulphuric acid) we duplicated the operations performed by Housley and Bentley in their treatment of those fractions of cabbage extract which contained IAN precursor. The details of this comparison are set out in the experimental section. Housley and Bentley fractionated cabbage extract into ether-soluble neutral, ether-soluble acidic, and water-soluble fractions by extraction with ether after pH adjustment. Their IAN precursor was found, by chromatography and bio-assay, mainly in the water-soluble, but also in the acidic ether-soluble, fraction. Elution of the IAN precursor from chromatograms and rechromatography led to a decrease in IAN precursor and an appearance of indolyl-3-acetonitrile. When we applied Housley and Bentley's procedures to indolyl-3-pyruvic acid oxime, results analogous in all particulars were obtained. In a number of their chromatograms Housley and Bentley detected two other growth active substances (R_f 0.10–0.20 and R_f 0.55–0.65, respectively, in 2-PrOH/NH_3). Chromatography of the ether extract of an acidified 5% $NaHCO_3$ solution of indolyl-3-pyruvic acid oxime gave, in addition to the expected spots of starting material and IAN, two weakly Ehrlich-positive spots of unknown nature with R_f ranges, 0.11–0.18 and 0.60–0.70, similar to those of Housley and Bentley. These growth active zones may thus have been due to artifacts derived from their acidic IAN precursor during extraction and chromatography.

The inhibition of IAN formation by dimedone, which has already been referred to, requires explanation. Since under mild conditions dimedone reacts specifically with aldehydes (e.g. ref. 32) it would appear that an aldehyde is implicated in IAN formation. This consideration led Gordon (14) to reject indolyl-3-pyruvic acid oxime as a possible intermediate in IAN biosynthesis in favor of indolyl-3-acetaldehyde. Preliminary experiments have now shown that Gordon's inference was based on insufficient evidence: whereas incubation of indolyl-3-pyruvic acid oxime at pH 2.0 and at pH 5.0, yielded IAN in 52.5% and 12.9% yield respectively (Table I), incubation of the oxime under similar conditions in the presence of dimedone did not yield detectable amounts of nitrile. Instead, the neutral ether-soluble fraction contained an oily yellow material of unknown structure which had no trace of –CN absorption at 2250 cm^{-1}, and showed, in addition to the indole bands at 280–288 mμ, an absorption band at 410 mμ, which disappeared in alkaline solution. Incubation of indolyl-3-pyruvic acid with dimedone, either in the presence or absence of hydroxylamine, gave rise to a similar yellow substance. When indolyl-3-acetonitrile was incubated with dimedone, starting material was recovered in 95% yield. Although the nature of the yellow dimedone adducts of indolyl-3-pyruvic acid and its oxime is as yet undetermined, it is clear that dimedone inhibits their conversion to indolyl-3-acetonitrile. This is consistent with Gordon's experimental results (14) on the inhibition of IAN formation from tryptophan in the presence of dimedone in *Avena* coleoptiles.

The auxin activity of indolyl-3-pyruvic acid oxime was compared with that of IAN and of indolyl-3-acetic acid in the *Avena* section (straight growth) test.[2] The oxime was found to have from 25% to 20% of the auxin activity of indolyl-3-acetic acid (compared

[2]*We are greatly indebted to Drs. J. Shen and S. A. Gordon, Argonne National Laboratory, Lemont, Illinois, for this study.*

on a molar basis) over a concentration range of 10^{-4} to 10^{-2} gram moles per liter (Fig. 1).

The chemical reactivity of indolyl-3-pyruvic acid oxime, the behavior of this substance on chromatography and solvent partition, and its biological activity thus correspond to the properties of the IAN precursor described by Housley and Bentley.

The evidence is compatible with the hypothesis that indolyl-3-pyruvic acid oxime is the biochemical precursor of indolyl-3-acetonitrile.

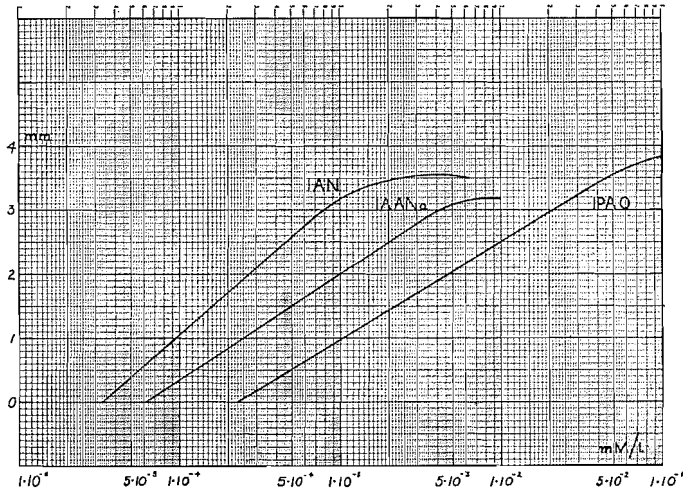


FIG. 1. Auxin activities of indolyl-3-acetonitrile (IAN), sodium indolyl-3-acetate (IAA.Na), and indolyl-3-pyruvic acid oxime (IPAO) in the *Avena* section (straight growth) test: 3-mm sections; medium: 0.01 *M* $NaHCO_3$, containing 2% w/v sucrose (2 ml); 20 hours.
Ordinate: mm increase over original length, corrected for control sections in medium alone (linear scale).
Abscissa: concentration of auxin in millimoles per liter (log scale).

EXPERIMENTAL

Indolyl-3-pyruvic acid was prepared according to Bentley *et al.* (5). Indolyl-3-pyruvic acid oxime was obtained from indolyl-3-pyruvic acid (25) and also by nitrosation of ethyl α-acetyl-β-(3-indolyl)-propionate (26), melting at 157°, pK' 3.4 (i.e., pH at half equivalence in saturated aqueous solution), infrared absorption (Nujol) (cm^{-1}): 3360(s), 1690(s), 1620(m); ultraviolet absorption (λ_{max}, mμ (log ϵ)): 225(4.13), 274(3.83), 282(3.84), 290(3.77).

Indolyl-3-acetonitrile

(1) *From Indolyl-3-pyruvic Acid*

Indolyl-3-pyruvic acid (0.400 g, 0.00197 mole) and hydroxylamine hydrochloride (0.136 g, 0.00197 mole) in 30 ml water were refluxed 2.5 hours. The solution, which contained a small amount of dark brown precipitate, was filtered, allowed to cool, and extracted with ether (5 times 10 ml). The ether extract was washed with 5% sodium bicarbonate solution and with water, dried over Na_2SO_4, and concentrated, yielding indolyl-3-acetonitrile as an oily residue. The product was distilled at 100° and 10^{-3} mm and was obtained as a yellow oil (0.184 g, 60%), whose infrared (in $CHCl_3$ (cm^{-1}): 3450(s), 2250(s), 1615(m), 1455(s), 1350(s), 1330(s)) and ultraviolet (λ_{max}, mμ (log ϵ) in MeOH: 220(4.50), 273(3.78), 279(3.80), 288(3.68)) absorption was identical with that of an authentic specimen melting at 35–36°, prepared from gramine methiodide (33). The oil crystallized on seeding with a few crystals of the authentic sample.

A small portion of the oily product was converted to the picrate, melting at 127–128° (33).

(2) *From Indolyl-3-pyruvic Acid Oxime*

(i) At 100°.—The oxime (0.100 g, 0.00046 mole) was dissolved in 20 ml 0.05 *M* H_2SO_4 (0.001 mole) and the solution refluxed 3 hours in an atmosphere of nitrogen. On cooling, a small amount of dark sediment was filtered off, the solution was extracted with ether, the ether extract washed with 5% bicarbonate solution and with water, dried over Na_2SO_4 and concentrated, and the oily residue distilled at 100° and 10^{-3} mm to yield 0.068 g (95%) indolyl-3-acetonitrile as a light yellow oil, crystallizing on seeding with an authentic specimen.

(ii) At 40°.—A solution of oxime (0.020 g, 0.000092 mole) in 20 ml buffer solution was allowed to stand 12 hours at room temperature and then 24 hours at 41.5°. Buffers used were those of Clark and Lubs, HCl/KCl (pH 1, 1.6, and 2), phthalate (pH 3, 4, and 5), and $H_2PO_4^-/HPO_4^=$ (pH 7). Some discoloration was observed at pH 1–4. The solution was extracted with ether; the extract washed with 5% bicarbonate and water, dried over Na_2SO_4, and concentrated; the residue was dissolved in $CHCl_3$ and identified as indolyl-3-acetonitrile by its infrared absorption. The yield was determined by measuring the intensity of the ultraviolet absorption at 288 mμ of the product in methanol at suitable dilution. Results are summarized in Table I. Incubation of the oxime in the presence of dimedone (0.200 g) was carried out similarly and the reaction mixture was worked up for neutral products in the same way, except that dilute NaOH, instead of $NaHCO_3$ solution, was used for washing, to remove unreacted dimedone.

Solvent Fractionation and Chromatography of Indolyl-3-pyruvic Acid Oxime

Chromatography.—Solvents used in ascending paper chromatography on Whatman No. 1 paper were (*a*) 2-propanol/0.15 *M* ammonia (1:4), and (*b*) 1-butanol, saturated with 1.5 *M* ammonia. Chromogenic reagents applied were (i) Salkowski reagent: ferric chloride/perchloric acid (100 ml 5% aqueous $HClO_4$ and 2 ml 0.05 *M* $FeCl_3$); (ii) nitrous acid/nitric acid (1 g KNO_2 in 200 ml HNO_3, sp. gr. 1.42, diluted 10 times); (iii) Ehrlich's reagent (2 g *p*-dimethylaminobenzaldehyde in a mixture of 80 ml absolute ethanol and 20 ml HCl, sp. gr. 1.18) (15).

Solvent fractionation of the oxime according to the procedure used by Housley and Bentley (15) for the investigation of cabbage extract.—For purposes of comparison Housley and Bentley's operations and results at each stage are quoted in square brackets.

Preliminary Fractionation

(A) Indolyl-3-pyruvic acid oxime (20 mg) in 15 ml 95% ethanol adjusted to pH 3.2 with 0.05 *M* H_2SO_4, was stirred at −10° for 40 hours (fraction 1). Chromatography in 2-PrOH/NH_3, R_f 0.42, i.e., unchanged oxime. [Frozen ground cabbage was extracted for 40 hours at −10° with 95% ethanol acidified to pH 3.2 with H_2SO_4. Not chromatographed.]

(B) The pH of the solution was adjusted to pH 5 by addition of saturated aqueous $Ba(OH)_2$, $BaSO_4$ was filtered off and ethanol removed from the filtrate *in vacuo* at 30° (fraction 2). Chromatography in 2-PrOH/NH_3 gave a spot at R_f 0.42, i.e., unchanged oxime. The residual solution was mixed with 3 ml phthalate buffer, pH 3, and allowed to stand at room temperature 24 hours. [$Ba(OH)_2$ solution was added adjusting the pH to pH 5, the extract was filtered, ethanol removed at 35° *in vacuo* and the pH of the residual solution adjusted to pH 3 with dilute H_2SO_4. Not chromatographed.]

(C) The solution was exhaustively extracted with ether, yielding ether extract (fraction 3) and aqueous layer (fraction 4). Chromatography of the ether layer in 2-PrOH/NH_3

gave spots R_f 0.42 (oxime) and R_f 0.90 (nitrile). The aqueous layer showed a spot at R_f 0.42 (oxime) only. [The solution was extracted with ether, yielding an ether-soluble fraction and a water-soluble fraction.]

The above experiments show that indolyl-3-pyruvic acid oxime is stable at pH 3.2–5 at −10°, but that it is partially converted to the nitrile at pH 3 and room temperature. They also show that the oxime is soluble both in ether and in water. Later steps were carried out with freshly prepared oxime in the requisite solvent.

Investigation of the "Ether-soluble" Fraction

(D) Indolyl-3-pyruvic acid oxime (10 mg) was dissolved in 5 ml ether giving a solution corresponding to fraction 3. This was extracted with 5% $NaHCO_3$ solution, yielding an ethereal and an aqueous layer. The ethereal layer was dried (fraction 6).

The aqueous bicarbonate layer was acidified to pH 3 with 0.05 M H_2SO_4. This solution was divided into two portions, one of which was extracted with ether immediately, and the ether layer washed and dried (fraction 5*a*), the other extracted with ether after 16 hours and the ether layer treated similarly (fraction 5*b*).

Chromatography of the "neutral" fraction 6 in 2-PrOH/NH_3 showed a single weak spot of R_f 0.90, i.e., indole-3-acetonitrile. The "acidic" fraction 5*b* showed an intense spot at R_f 0.90 (indole-3-acetonitrile), and a weak spot at R_f 0.41 (oxime). Fraction 5*a* showed a strong spot at R_f 0.90 (nitrile), a strong spot at R_f 0.42 (oxime), and in addition weakly Ehrlich-positive spots at R_f 0.60–0.70 (green, changing to yellow, changing to green), R_f 0.42–0.55 (purple, changing to green), and R_f 0.11–0.18 (blue green).

Evidently prolonged exposure to pH 3 had caused almost complete conversion of oxime to nitrile (fraction 5*b*), whereas during shorter contact with an acid medium (fraction 5*a*) conversion of oxime to nitrile was incomplete. The nature of the materials giving rise to the additional indolic spots in this fraction is obscure, but it is suggestive that Housley and Bentley report the occurrence, in a number of their chromatograms, of growth active materials of R_f 0.1–0.2 and 0.5–0.7 which could not be ascribed to recognized auxins. It is possible that these materials may have been breakdown products of their precursor, similar to the oxime breakdown products of fraction 5*a*. [The ether-soluble fraction from step C was washed, dried, concentrated, redissolved in ether, and extracted with bicarbonate solution. The ether layer gave a neutral fraction, containing growth active zones at R_f 0.46–0.90 (mainly IAN, but presumably some precursor, due to incomplete fractionation), whereas the bicarbonate layer, after acidification to pH 3, extraction into ether, and drying and evaporation of the ether layer, showed weak auxin activity at R_f 0.25–0.50 (precursor) and strong activity at R_f 0.54–0.89 (IAN).]

Investigation of the "Water-soluble" Fraction

(E) Indolyl-3-pyruvic acid oxime (10 mg) was dissolved in 5 ml water, giving a solution corresponding to fraction 4 (step C). Chromatography in 2-PrOH/NH_3 gave R_f 0.42 only. The area of the paper corresponding to the oxime was eluted with 4 ml water, and the eluate concentrated and rechromatographed with 2-PrOH/NH_3 and also with 1-BuOH/NH_3. The former chromatogram showed spots at R_f 0.42 (oxime) and R_f 0.90 (nitrile), the latter at R_f 0.16 (oxime) and R_f 0.90 (nitrile). During elution and rechromatography oxime had thus partially decomposed to nitrile. [The pH of the water-soluble fraction from step (C) was adjusted to pH 5.3 and the solution concentrated at 25°. Chromatography in 2-PrOH/NH_3 of the concentrate gave a precursor spot at R_f 0.4–0.5 and an IAN spot at R_f 0.8–0.9, the latter presumably due to previous acid treatment. The area on the chromatogram corresponding to precursor was eluted with 4 ml water at 5° and

the eluate rechromatographed with 2-PrOH/NH_3, where it showed R_f 0.4–0.5, and with *n*-BuOH/NH_3, where it gave R_f 0–0.15. In both the latter chromatograms an IAN spot at R_f 0.8–0.9 also appeared. Thus during elution and rechromatography precursor had partially decomposed to IAN.]

(F) Indolyl-3-pyruvic acid oxime (10 mg) was dissolved in 3 ml water and heated under reflux 25 minutes. On cooling, the solution was extracted with ether. Chromatography of the ether extract in 2-PrOH/NH_3 gave spots at R_f 0.42 (oxime) and R_f 0.90 (nitrile). Chromatography of the aqueous layer gave a weak spot, R_f 0.42 (oxime). [The pH of the water-soluble fraction from step C was adjusted to pH 5.3, the solution was concentrated at 25° and heated at 98–100° for 25 minutes, cooled, dissolved in 20 ml aqueous $NaHCO_3$, and the solution extracted with ether. Chromatography of the ethereal "neutral" fraction showed a weak auxin zone at R_f 0.29–0.44 (precursor which according to Housley and Bentley had entered this fraction due to incomplete separation) and a strong zone at R_f 0.7–0.95 (nitrile). Elution and rechromatography in 2-PrOH/NH_3 of the zone R_f 0.29–0.44 gave rise to more IAN. The aqueous phase was acidified to pH 3 and extracted with ether, yielding an "acidic" fraction, which on chromatography showed auxin activity at R_f 0.25–1.00 with activity peaks at R_f 0.85–0.92 (IAN, presumably derived from precursor due to acidification during fractionation) and at R_f 0.35–0.48 (presumably precursor, but ascribed by Housley and Bentley to indolyl-3-acetic acid, since this region gave a pink color with Salkowski's reagent).]

ACKNOWLEDGMENTS

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