64°, 85.5–86°[13]), were prepared by the method of Backer and Beute[13] from 2-propynoic acid.

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(13) H. J. Backer and A. E. Beute, *Rec. trav. chim.*, **54**, 167 (1935).

(14) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

The Detection of 3-Indoleacetic Acid in Cauliflower Heads. Chromatographic Behavior of Some Indole Compounds[1,2]

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Introduction

In 1949 Wittwer[3] ascertained that both plant growth inhibitors and growth stimulators occurred in the ether extract of cauliflower heads with a predominance of the growth inhibitor. Luckwill[4] made the same observation in other plant tissue and also found two "auxins" and one inhibitor present in young broccoli leaves. Bennet-Clark and Ball[5] postulated a possible mechanism of action between growth inhibitors and growth stimulators in plants.

Holley, *et al.*,[6] isolated from the acid fraction of an ether extract of cabbage a substance without biological activity but giving a positive test with the Tang and Bonner reagent.[7] They indicated that 3-indoleacetic acid was responsible for most of the activity along with two other unidentified biologically active substances. Jones, *et al.*,[8] identified 3-indoleacetonitrile in the neutral fraction of an ether extract of cabbage. More recently, Bennet-Clark and Kefford[9] have suggested the general occurrence of several growth regulators in the ether extract of broad bean, peas, sunflower, corn and potatoes and identified one of them as 3-indoleacetic acid.

This communication reports the identification of 3-indoleacetic acid in cauliflower heads by chromatographic techniques and the R_f values of several related indole compounds.

Experimental

Colorimetric Assay of 3-Indoleacetic Acid.—One kg. of an ethanol extract representing 20 kg. of fresh cauliflower heads (Snowball X) was diluted with one l. of water, acidified with orthophosphoric acid and extracted with ethyl acetate. This extract was shown to contain 7 mg. of 3-indoleacetic acid by the Gordon and Weber procedure.[10] However, later work, reported herein, indicated that most of the color produced in the colorimetric assay did not result from 3-indoleacetic acid but from other compounds probably containing an indole nucleus. As a consequence, an attempt to isolate 3-indoleacetic acid as the *sym*-trinitrobenzene adduct,[11] based on the presence of 7 mg. of acid, was not successful since so small an amount of adduct was obtained that purification was not satisfactory. This lack of specificity of existing colorimetric procedures for 3-indoleacetic acid has been recognized earlier[6,10] and more recently investigated in this Laboratory.[12]

Detection of 3-Indoleacetic Acid.—Thirty kg. of fresh cauliflower heads was successively extracted four times with 5-l. portions of peroxide-free ether. Upon removal of the ether *in vacuo* 30 g. of residue was obtained. One g. of this residue was dissolved in 25 ml. of ethyl acetate and extracted with a 1% solution of sodium bicarbonate until the aqueous solution remained alkaline following extraction. The combined aqueous extracts were acidified with orthophosphoric acid and then re-extracted with ethyl acetate. This extract was placed on a line on a 20 × 30 cm. sheet of Whatman No. 1 filter paper and chromatographed using a solution of 1-propanol–1.5 *N* ammonium hydroxide–water (60:30:10 v./v.) and employing the descending technique. The location of the indole compounds on the chromatogram was determined by spraying with a solution of 1 g. of *p*-dimethylaminobenzaldehyde, 10 ml. of concentrated hydrochloric acid and 90 ml. of ethanol. The indole compounds in general produce a red to purple coloration. The area between the R_f values of 0.60–0.90 which contained all the colored zones, as determined by developing a center strip, was eluted with acetone. A portion of the resulting material was rechromatographed as a spot using the same technique

TABLE I

R_f VALUES OF INDOLE COMPOUNDS

(R_f values × 10^2), temp., 30°

Compound	Solvent: 1-Butanol sat. with 5% NH_4OH	Solvent: 1-Propanol–concd. NH_4OH–H_2O (60–30–10 v./v.)	Color of spot with *p*-dimethylaminobenzaldehyde
3-Indoleacetic acid	25	75	Purple
3-Indoleacetonitrile	85	89	Purple
3-Indoleacetaldehyde[a]	88	88	Yellow brown (streak)
3-Indoleacetohydrazide	91	69	Purple
3-Indoleacetohydroxamic acid	58	71	Yellow
3-Indoleacetamide	84	87	Blue-purple
3-Indolepropionic acid	30	77	Purple
3-Indolebutyric acid	37	84	Purple
3-Indolecarboxaldehyde	87	87	Purple
3-Indolecarboxylic acid	15	68	Pink
Ethyl 3-indoleacetate	84	89	Purple
Ethyl 3-indolecarboxylate	88	96	Yellow
2-Phenyl-3-indoleacetic acid	90	88	Yellow-purple
2-Methyl-3-indoleacetic acid	26	78	Purple
2-Methylindole	86	96	Red
Tryptophol	88	90	Purple
L-Tryptophan	23	74	Purple
Tryptamine	79	90	Purple
1-Hydroxyl-3-indoleacetic acid	22	97	Brown
N,N'-Diindolyl-3,3'-diacetic acid	24	80	Brown
Indole	95	95	Pink
Isatin	74	80	Yellow

[a] Obtained as the sodium bisulfite addition product from Dr. Reed Gray, Pineapple Research Institute, Honolulu, Hawaii.

(1) Journal Article No. 1547 from the Michigan Agricultural Experiment Station, Michigan State College, East Lansing, Michigan.

(2) This research was supported by the Horace H. Rackam Research Endowment.

(3) S. H. Wittwer, unpublished work, 1949.

(4) L. C. Luckwill, *Nature*, **169**, 375 (1952).

(5) T. A. Bennet-Clark and N. G. Ball, *J. Exp. Bot.*, **2**, 169 (1951).

(6) R. W. Holley, F. P. Boyle, H. K. Durfee and A. D. Holley, *Arch. Biochem. Biophys.*, **32**, 192 (1951).

(7) Y. W. Tang and J. Bonner, *Arch. Biochem.*, **13**, 11 (1947).

(8) E. R. H. Jones, H. B. Henbest, G. F. Smith and J. A. Bently, *Nature*, **169**, 485 (1952).

(9) T. A. Bennet-Clark and N. P. Kefford, *ibid.*, **171**, 645 (1953).

(10) S. A. Gordon and R. P. Weber, *Plant Physiol.*, **26**, 192 (1951).

(11) C. T. Redemann, S. H. Wittwer and H. M. Sell, THIS JOURNAL, **73**, 2957 (1951).

(12) Wm. Houff, O. N. Hinsvark, L. E. Weller, S. H. Wittwer and H. M. Sell, paper presented before the Division of Organic Chemistry at the American Chemical Society Meeting, Chicago, Ill., Sept., 1953.

as previously described. Upon subsequent development of the chromatogram, three distinct spots were obtained having the following R_f values: 0.62–0.64, 0.75–0.77 and 0.86–0.88. When 5 micrograms of 3-indoleacetic acid was added to a like portion of the residue and chromatographed, the same three spots were evident with an enrichment of the colored zone having an R_f value of 0.75–0.77. A parallel chromatogram of known 3-indoleacetic acid gave an R_f value of 0.75. An acid corresponding to an R_f value of 0.75–0.77 was eluted from the papergram and applied in the form of a lanolin paste to the first internode of red kidney bean seedlings. A pronounced negative curvature was obtained. Elution of a like area of a chromatogram and treatment with the Gordon and Weber reagent[10] gave a colored product having a maximum absorption at 540 mμ. These properties described for the spot having an R_f value of 0.75–0.77 are in agreement with those of 3-indoleacetic acid.

Two other spots were obtained on the chromatogram having R_f values of 0.86 to 0.88 and 0.62–0.64. The substance having an R_f value 0.86–0.88 produced a negative curvature when applied to the first internode of bean seedlings while the substance present in the greatest amount having an R_f value of 0.62–0.64 showed only slight biological activity. In an effort to identify these substances a number of indole derivatives were chromatographed to find known compounds which would migrate at the rate of the unknowns. The R_f values of these substances are given in Table I.

It is evident from Table I that a number of known indole compounds migrate within the range of R_f values 0.86–0.88. However, with one exception, these are neutral compounds not generally found in appreciable amounts in an acid fraction. Bennet-Clark and Kefford[9] have reported that 3-indoleacetonitrile, although not an acid, is carried to some extent from an ether solution into sodium bicarbonate solution and back into ether on re-acidification. However, using an ethyl acetate solution of synthetic 3-indoleacetonitrile and mutually saturated solutions for extracting, with washing between extractions, extremely small amounts of 3-indoleacetonitrile were obtained.

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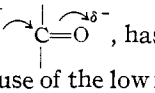
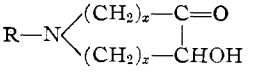
COMMUNICATIONS TO THE EDITOR

CYCLIC AMINOACYLOINS. RING-SIZE LIMITATION OF TRANSANNULAR INTERACTION BETWEEN N AND CO

Sir:

A transannular amide-type neutralization,  , has been proposed by Robinson[1] as the cause of the low frequency observed for the infrared absorption of the carbonyl group in the alkaloid cryptopine (10-membered ring) and also of the ketonic groups in N-methylpseudostrychnine and N-methylpseudostrychnidine (9-membered rings).[2]



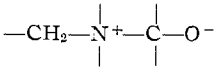
	R	x	Infrared absorption maxima, cm.$^{-1}$ Base	Perchlorate
a	C_6H_5	2	1701 3458	
b	CH_3	3	1666 3410	3440
c	C_2H_5	3	1671 3428	3425
d	CH_3	4	1700 3462	1710 3480
e	C_2H_5	4	1705 3470	
f	CH_3	3, no CHOH	1683	3380

We find that the infrared data accumulated in this Laboratory for a series of related compounds,[3] which are structurally much simpler than the alkaloid products, are consistent with the hypotheses of transannular interaction of RN< and >C=O. Moreover, we are able to assign the probable limits of ring size within which appreciable transannular interaction of these groups will occur. We have also innovated the comparison of the apparent dissociation constants of a cyclic aminoketone in solvents of different dielectric strength as a diagnostic tool for demonstrating transannular interaction.

1-Alkyl-1-azacycloalkanolones of type I (odd-membered rings) have been synthesized *via* the acyloin condensation of the corresponding aminodiesters, which were made by treatment of the primary amine with the appropriate ω-haloester. The acyloins of ring size: 11, 13, 15, 17, 19 and 23 members possess normal ketone carbonyl absorption in the infrared (1700–1713 cm.$^{-1}$), as does Ia, a 7-membered ring, while the two 9-membered ring examples (Ib, c) have infrared maxima in the 6 μ region which are at abnormally low frequency for C=O stretching in a saturated ketone. The perchlorate of the 11-membered ring (Id) shows both ketone and O–H/N–H absorption, whereas the perchlorate of the 9-membered ring (Ib, also Ic) is transparent in the 6 μ region (no C=O). The contrast between the 11- and 9-membered ring com-

(1) F. A. L. Anet, A. S. Bailey and Sir Robert Robinson, *Chemistry and Industry*, 944 (1953); also, Sir Robert Robinson in the Karl Folkers Lectures at the University of Illinois, September 29, 30, October 1, 1953. E. H. Mottus, H. Schwarz and L. Marion, *Can. J. Chem.*, **31**, 1144 (1953), have come to a similar conclusion on the basis of spectral studies on protopine.

(2) R. Huisgen, H. Wieland and H. Eder, *Ann.*, **561**, 193 (1949), suggested the contribution of a limiting electronic species,



to the structure of vomicine (9-membered ring) to account for the unusual chemical behavior of the tertiary amino and ketonic groupings in this alkaloid.

(3) R. C. Fox, Ph.D. thesis, University of Illinois, 1953.

(4) We wish to thank Miss Helen Miklas for determination of the infrared absorption spectra. The bases were determined in carbon tetrachloride solution; the salts, in Nujol mull. Where the entry is blank, the infrared spectrum was not obtained.