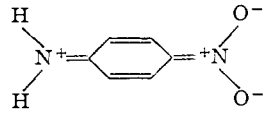
spectral bands. Accordingly, we cannot be certain that the observed band near 6.7 μ corresponds to quite the same stretching mode in all cases.

One conclusion does seem possible, however. The 6.6–6.7 μ band in compounds like *p*-nitroaniline, *p*-nitrophenol and *p*-nitroanisole must correspond to C–C stretching parallel to the main axis of the molecule. This is indicated by the great intensity of the band in these compounds, which suggests vibration parallel to the dipole, and the fact that no interaction occurs between this vibration and the NO_2 asymmetrical stretching vibration, even when the frequencies coincide, as they do in *p*-nitroaniline (see Fig. 2). The shift of the band to shorter wave lengths by substituents having more negative σ-values indicates an increase in the order of the bonds parallel to the molecular axis, and is in accord with the view that resonance forms such as V are contributing to the over-all structure of these molecules.



V

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[CONTRIBUTION FROM THE BOTANY DEPARTMENT, CORNELL UNIVERSITY]

The Identification of Compound A from Coconut Milk as 1,3-Diphenylurea[1]

BY E. M. SHANTZ AND F. C. STEWARD

RECEIVED MAY 7, 1955

Compound A from coconut milk has been reisolated and identified as 1,3-diphenylurea, identical with a synthetic sample in its physical, chemical, and biological properties. In combination with casein hydrolysate, it promotes growth by cell division in cultures of carrot phloem tissue in a manner similar to coconut milk but to a lesser extent. The degree of response is variable, being dependent upon inherent characteristics of the individual carrot root from which the explants are taken. The chemical determination of the probable maximum level of anilide in a different sample of coconut milk is also reported.

In the attempt to determine the chemical nature of the substances in coconut milk responsible for the stimulation of growth in mature carrot phloem cells, Shantz and Steward[2] described the isolation and general characteristics of three crystalline compounds, then designated as A, B and C. Compound A has now been reisolated in larger amount and identified as 1,3-diphenylurea, identical in both chemical and biological properties with a synthetic sample.

The first small isolate of compound A was obtained as a crude crystalline product when an alcoholic extract of the mercury-freed precipitate from approximately 700 gallons of coconut milk, that had been treated in 50% alcohol with mercuric acetate, was concentrated and filtered. The second isolation of compound A in larger amount was made from the neutral fraction of the ether extract from the above filtrate. This fraction (72 g.) was dissolved in 500 ml. of petroleum ether (b.p. 60–70°) and seeded with a fraction of a mg. of compound A crystals previously obtained. The solution was filtered after storage at room temperature for 7 days, leaving a residue of 480 mg. of light brown needles. No further crystalline material could be obtained even after concentration to 200 ml. and prolonged storage at 5°. The crude crystalline product was recrystallized three times from hot absolute ethanol to give a final yield of 317 mg. of fine white needles.

Analyses[3] on the isolated product gave: C, 73.58; H, 5.78; O, 7.63, N, 13.17; mol. wt. between 173 and 230 as determined by isothermal distillation with acetone. Required for $C_{13}H_{12}N_2O$: C, 73.57, H, 5.70, O, 7.54; N, 13.19; mol. wt., 212. The melting points of the isolated compound A (241–243.5°) and synthetic 1,3-diphenylurea (242–244.5°) showed no depression on mixing (242–244°). Complete infrared scans (potassium bromide disc method) of the natural compound A and synthetic 1,3-diphenylurea were identical, as were the ultraviolet absorption spectra in methanol and in ethanol.

The ability of compound A to induce growth in carrot explants was originally tested by methods adopted in this Laboratory[4] using explants from a number of different roots. Although the magnitude of the growth response was to a large extent a function of the individual carrot roots, activity was demonstrated in most of the experiments carried out at that time.[5] The addition of compound A at about 1 p.p.m. to the basal medium had but little effect on growth, but if casein hydrolysate were also added, the growth response frequently approached that obtained by the addition of whole coconut milk. The criterion of growth in these experiments was the increase in fresh weight shown by the cultured explants. By maceration and cell counting techniques, to be described elsewhere, it is now pos-

(1) This work has been supported throughout by grants C-1357(C2) to C-1357(C4) to F.C.S. from the National Cancer Institute, National Institutes of Health of the U. S. Department of Health, Education, and Welfare. E.M.S. has been working at the Botany Department of Cornell University as a member of the Sloan-Kettering Institute for Cancer Research.

(2) E. M. Shantz and F. C. Steward, THIS JOURNAL, **74**, 6133 (1952).

(3) The authors gratefully acknowledge the assistance of Drs. R. H. Wallick, J. J. Kirkland, R. L. Dalton, and other members of the Technical Division of the Grasselli Chemicals Department of E. I. du Pont de Nemours & Company in obtaining the analytical data.

(4) F. C. Steward, S. M. Caplin and F. K. Millar, *Ann. Bot. N.S.*, **16**, 57 (1952).

(5) F. C. Steward and E. M. Shantz, *Année Biol.*, **30**, 399 (1954).

TABLE I

EFFECT OF 1,3-DIPHENYLUREA (DPU) ON GROWTH OF CARROT TISSUE EXPLANTS[a]

Initial wt. of explants = 2.3 mg.

Treatment	Carrot	Mg. fresh wt./culture A (9 days)	B (17 days)	C (17 days)	D (18 days)	Cells/culture × 10^{-4} A	B	C	D
Basal medium		6.2	7.2	4.6	9.4	34.1	45.2	28.8	35.6
Basal + 2.0 p.p.m. DPU		10.2	9.4	7.5	10.2	73.9	50.2	49.0	38.8
Basal + coconut milk		52.3	148	220	129	1300	2410	3020	
Basal + casein hydrolysate		11.7	29.4	64.8	17.6	39.1	201	536	88.5
Basal + cas. hyd. + 2.0 p.p.m. DPU		13.8	56.8	109	25.8	68.3	509	1040	164
Basal + cas. hyd. + coconut milk		40.9	294	323	173	826	2690	2690	1720

[a] Each figure represents the average value of 4–9 replicate explants per treatment.

sible to estimate the number and average size of cells per explant before and after growth in various media.

In more recent growth experiments carried out with both synthetic 1,3-diphenylurea and the product isolated from coconut milk the growth responses have been generally lower than those reported earlier. In particular, explants from carrot roots that had been in storage for a number of months appeared much less responsive to 1,3-diphenylurea than did those from certain freshly harvested strains. In all cases, however, parallel results were obtained with the synthetic compound and the natural isolate. Although the carrot explants usually gave a significant response to diphenylurea only in the presence of casein hydrolysate, they did not require the presence of an auxin-like compound such as indoleacetic acid or 2,4-D in the medium.

Representative data on growth responses by explants from 4 different strains of carrot roots are shown in Table I. Carrot A had been stored for 6 months while carrots B, C and D were from three freshly harvested varieties grown in the 1954 variety trials at the New York Experiment Station, Geneva. The results are given both as average final fresh weight per culture and as average total number of cells per culture. Calculation of the average cell size, which can be done readily from the data in the table, shows that diphenylurea tends to increase the number of cells rather than cell size, somewhat in the manner of whole coconut milk but to a lesser degree. In the more favorable cases (*e.g.*, carrot C) the combination of diphenylurea and casein hydrolysate accounted for half the growth induced by whole coconut milk and a third of the growth induced by coconut milk plus casein hydrolysate. Among the numerous growth experiments not reported here, diphenylurea has been found in some cases to be without effect and in many instances to have relatively small effects which are significant, however, when tested by appropriate statistical procedures. It is thus apparent that many carrot roots yield explants that respond to the whole coconut milk complex but not to diphenylurea, due to inherent variables in the individual roots which are as yet not understood. Diphenylurea has also been tested with significant positive results, to be presented elsewhere, for its ability to induce growth in potato tuber explants (which also require 2,4-D[6] or a similar substance[7]) and in artichoke tuber explants.

(6) F. C. Steward and S. M. Caplin, *Science*, **113**, 518 (1951).

(7) E. M. Shantz, F. C. Steward, M. S. Smith and R. L. Wain, *Ann. Bot. N.S.*, **19**, 49 (1955).

Though urea[8] and certain ureides[9,10] have been shown to occur in plants, diphenylurea seems not to have been reported previously. Some earlier references to its action in growth tests do exist, however. Thompson, Swanson and Norman[11] tested 1,3-diphenylurea amongst some 1000 organic compounds and found it to be 50% as effective as 2,4-D when applied to kidney bean leaves. It is also said to inhibit nitrogen fixation by Azotobacter.[12]

The identification of compound A from coconut milk as 1,3-diphenylurea raises the question of whether this substance does in fact occur as such *in vivo*. Tests by methods[13,14] for the direct determination of substances that yield aromatic amines on treatment with alkali showed concentrations considerably lower than those expected from the amount that was isolated. These tests were carried out on whole coconut milk from a different source than that originally used and on a butanol extract of this material. The tests on whole coconut milk solids showed about 1 p.p.m. and the butanol extract about 14 p.p.m. of diphenylurea, on the assumption that the reacting material was actually this compound. This was only about $^1/_5$ of the predicted concentration as calculated from the amount isolated by the mercury precipitation procedure, though from a different source of coconut milk.

Thus, as in all procedures involving apparatus on the pilot plant scale and large amounts of solvents and reagents, the risk of contamination, however unlikely, must be considered. It would be a coincidence indeed if a contaminant were an active substance accompanying other substances active in the growth test, and there is, in fact, no evidence that such contamination did occur. The evidence for the natural occurrence of 1,3-diphenylurea as such in the intact nut would be strengthened by a further isolation by procedures other than those which led to its first isolation as compound A, but this has not yet been attempted on the large scale that would be necessary. These cautious reservations do not, however, affect the undoubted ability of the isolated substance to promote cell division in the same manner as coconut milk though not to the same extent.

It is now known that coconut milk and morpho-

(8) I. Reifer and J. Melville, *J. Biol. Chem.*, **178**, 715 (1949).

(9) R. Echevin and A. Brunel, *Compt. rend.*, **205**, 294 (1937).

(10) A. Brunel and G. Capelle, *Bull. soc. chim. biol.*, **29**, 427 (1947).

(11) H. E. Thompson, C. P. Swanson and A. G. Norman, *Bot. Gaz.*, **107**, 476 (1946).

(12) M. V. Federov, *Mikrobiologiya*, **15**, 23 (1946).

(13) W. E. Bleidner, H. M. Baker, M. Levitsky and W. K. Lowen, *Agric. Food Chem.*, **2**, 476 (1954).

(14) W. E. Bleidner, *ibid.*, **2**, 682 (1954).

logically similar materials may owe much of their growth-promoting properties to certain nitrogen-free substances not hitherto recognized as being active in this manner. Bioassays carried out in this Laboratory have also disclosed activity in certain other substances, both natural and synthetic. The recent isolation and identification of "kinetin" as 6-furfurylaminopurine by Miller, *et al.*,[15] adds a compound of a still different structural type to the growing list of such active substances. Detailed data on the biological responses to all of the above-mentioned substances, which bear no obvious chemical relationship to one another, will be presented elsewhere. However, their effects on the growth of various plant tissue cultures and their obvious interactions with casein hydrolysate, natural hormones such as indoleacetic acid, and synthetic growth regulators of the general type of 2,4-D, all emphasize that no single substance is the sole answer to the chemical induction of growth by cell division in plants.

ITHACA, N.Y.

(15) C. O. Miller, F. Skoog, F. S. Okumura, M. H. Von Saltza and F. M. Strong, THIS JOURNAL, **77**, 2662 (1955).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE FLORIDA STATE UNIVERSITY]

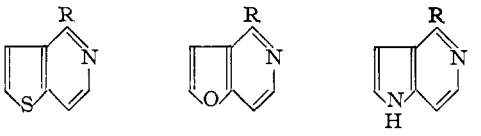
Pyrrolo[3,2-c]pyridines[1]

BY WERNER HERZ AND STANLEY TOCKER

RECEIVED MAY 31, 1955

The Bischler–Napieralski reaction has been applied successfully to derivatives of 2-(2-pyrrole)-ethylamine. The resulting dihydropyrrolo[3,2-c]pyridines were aromatized and their reduction to tetrahydro derivatives was accomplished. N-Formyl- and N-homoveratroyl-2-(2-pyrrole)-ethylamine could not be cyclized.

Earlier papers[2,3] of this series showed that sulfur (I) and oxygen (II) analogs of isoquinolines could be prepared by suitable modifications of the Bischler–Napieralski reaction from derivatives of thiophene and furan. In this paper we report the synthesis of the corresponding nitrogen analogs (III), pyrrolo[3,2-c]pyridines or 5-azaindoles.

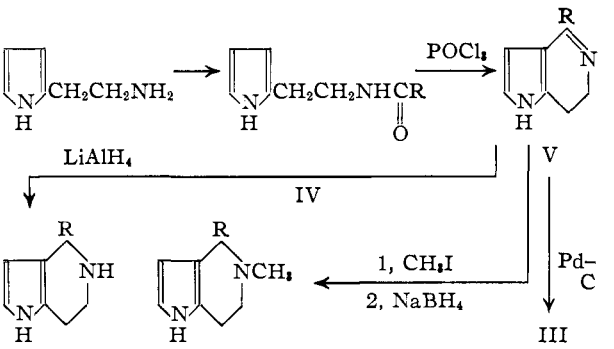


I II III

The only previously reported representative of this class of compounds, 6-methylpyrrolo[3,2-c]pyridine (2-methyl-5-azaindole), was prepared by Clemo and Swan[4] in 1% yield through the Madelung cyclization of 4-acetamido-3-picoline. Their efforts to synthesize 5-azaindole itself were unsuccessful. Our experience with N-acyl-2-(2-furyl)-ethylamines[3] and the availability of 2-(2-pyrrole)-ethylamines[5] suggested the possibility of synthesizing pyrrolo[3,2-c]pyridines by the Bischler–Napieralski method.

The sequence of reactions leading to pyrrolo[3,2-c]pyridines (III) is illustrated in the chart.

Cyclizations with phosphorus oxychloride in toluene were carried out in the mannner described for furan derivatives.[3] N-Benzoyl-2-(2-pyrrole)-ethylamine and the N-acetyl homolog were cyclized to 1-phenyl- and 1-methyl-3,4-dihydropyrrolo[3,2-c]-pyridines (V, R = phenyl, CH_3) in 24 and 18% yield while the N-homoveratroyl- and N-formyl-amides gave only amorphous solids which could not be purified sufficiently for elemental analysis. These yields are somewhat lower than the ones obtained in the thiophene and furan series.



R = H, CH_3, phenyl, 3,4-dimethoxybenzyl

Dehydrogenation of V (R = CH_3 and phenyl) to the completely aromatic 1-substituted pyrrolo[3,2-c]pyridines (III, R = CH_3 and phenyl) was accomplished in good yield by refluxing with palladium-on-charcoal in toluene solution. Spectroscopic evidence for successful aromatization is the observation that the second absorption band of III (R = CH_3) occurs at somewhat longer wave lengths (λ_{max} 220 and 272 mμ, λ_{min} 236 mμ; log ϵ_{max} 4.90 and 4.05, log ϵ_{min} 3.65) than the second band of V (R = CH_3) (λ_{max} at 222 and 260 mμ, λ_{min} 241 mμ; log ϵ_{max} 4.88 and 3.80, log ϵ_{min} 3.6).[6] Furthermore there is a pronounced similarity in the spectra of III, indole[7] and 7-azaindole[8] which supports the postulated structures.

1-Phenyl- and 1-methyl-3,4-dihydropyrrolo(3,2-c)pyridine were reduced by lithium aluminum hy-

(1) Supported in part by Grant RC-3097 from the United States Public Health Service, Department of Health, Education and Welfare.

(2) W. Herz, THIS JOURNAL, **73**, 351 (1951); W. Herz and Lin Tsai, *ibid.*, **77**, 3529 (1955).

(3) W. Herz and S. Tocker, *ibid.*, **77**, 3554 (1955).

(4) G. R. Clemo and G. A. Swan, *J. Chem. Soc.*, 198 (1948).

(5) W. Herz, THIS JOURNAL, **75**, 483 (1953).

(6) The spectra were determined in 95% ethanol and are very similar to those of the corresponding thieno[2,3]- and furano(3,2-c)pyridines.

(7) R. A. Friedel and M. Orchin, "Ultraviolet Spectra of Aromatic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1951.

(8) M. E. Robison and B. L. Robison, THIS JOURNAL, **77**, 457 (1955).