solution. The free semicarbazide was collected by filtration and recrystallized from absolute ethanol; yield 60%, m.p. 83–84°.

Anal. Calcd. for $C_{19}H_{41}N_3O$: C, 69.72; H, 12.53; N, 12.84. Found: C, 69.46; H, 12.28; N, 12.80.

Preparation of 4-Octadecylsemicarbazide (III). Ethyl Octadecylaminecarbamate.—To a well stirred ice cold suspension of octadecylamine (13.5 g., 0.05 mole) in 125 ml. of ether, contained in a three-neck flask, ethyl chloroformate (2.7 g., 0.025 mole) in 15 ml. of ether was added over a period of 30 minutes. To this mixture a solution of sodium hydroxide (2 g. in 35 ml. of water) and a solution of ethyl chloroformate (2.7 g. in 15 ml. of ether) were added simultaneously over a period of 1.5 hours. The ice-bath was removed and the mixture allowed to react for a further hour, at the end of which time a clear ether layer was obtained. The ether layer was washed with 10% sodium carbonate, water, dried over sodium sulfate and evaporated to dryness. The residue 13.3 g., m.p. 66–67°, was recrystallized from ethanol and pure material (m.p. 67–68°) was obtained as plates, which changed to needles at 60°.

Anal. Calcd. for $C_{21}H_{43}NO_2$: C, 73.84; H, 12.68; N, 4.10. Found: C, 74.32; H, 12.60; N, 4.11.

A sealed tube containing 3.0 g. of the above carbonate, 0.43 ml. of anhydrous hydrazine and 5 ml. of absolute alcohol was heated over a period of 22 hours at 130°. The residue after removal of excess hydrazine and alcohol was treated with ether and the ether insoluble material, Compound III, 0.26 g., m.p. 102–103°, was collected and recrystallized from ethanol; m.p. 104–106°.

Anal. Calcd. for $C_{19}H_{41}N_3O$: C, 69.67; H, 12.62; N, 12.83. Found: C, 69.60; H, 12.20; N, 12.62.

Dry HCl was passed through the dried ether layer and the precipitated hydrochloride (0.6 g., m.p. 103–115°) yielded after many recrystallizations from ethanol a small amount of octadecylamine hydrochloride (m.p. 159–161°) and mixed melting point with an authentic sample (m.p. 162–163°) showed no depression.

Anal. Calcd. for $C_{18}H_{40}NCl$: N, 4.58. Found: N, 4.48.

The final ether solution, after washing with 10% NaOH, water and drying over sodium sulfate yielded on evaporation, 1.8 g. of the original carbamate (m.p. 67–68°).

All the derivatives listed below were prepared in essentially the same manner, except as indicated, and the procedure is illustrated by the following example.

Heptadecylsemicarbazone of Benzaldehyde.—A solution of heptadecylsemicarbazide (1.0 g., 0.003 mole) and benzaldehyde (0.67 g., 0.006 mole) in 25 ml. of methanol containing two drops of glacial acetic acid was heated for a short time on a steam-bath and allowed to stand overnight. The crystalline precipitate was recrystallized from methanol and melted at 79–79.5°, yield 1.0 g., λ_{max} 285 mμ, ϵ_{max} 19,500.

Anal. Calcd. for $C_{25}H_{43}N_3O$: C, 74.76; H, 10.79; N, 10.47. Found: C, 75.53; H, 11.00; N, 10.36.

The semicarbazone of benzaldehyde shows λ_{max} 282 mμ, ϵ_{max} 24,000.

4-Pentadecylsemicarbazone of benzaldehyde after recrystallization from ethanol melted at 73–74°.

Anal. Calcd. for $C_{23}H_{39}N_3O$: C, 73.95; H, 10.50; N, 11.25. Found: C, 74.03; H, 10.17; N, 10.90.

2-Methyl-4-heptadecylsemicarbazone of benzaldehyde after recrystallization from methanol melted at 100–101°, λ_{max} 293 mμ, ϵ_{max} 20,800.

Anal. Calcd. for $C_{26}H_{45}N_3O$: C, 75.13; H, 10.91; N, 10.11. Found: C, 75.51; H, 10.86; N, 10.31.

Heptadecylsemicarbazone of Formaldehyde.—The crude product was dissolved in ether, filtered from insoluble purity, and the ether residue recrystallized several times from methanol and dried *in vacuo* over P_2O_5. Five grams of compound IV yielded 2.5 g. of the pure semicarbazone derivative, m.p. 66–68°.

Anal. Calcd. for $C_{19}H_{39}N_3O$: C, 70.09; H, 12.08; N, 12.92. Found: C, 70.18; H, 11.79; N, 12.68.

The above material on hydrogenation in glacial acetic acid with platinum oxide as catalyst absorbed the equivalent of one mole of hydrogen. However, the sought-for 1-methyl-4-heptadecylsemicarbazide could not be isolated in any state of purity.

4-Heptadecylsemicarbazone of cyclohexanone after recrystallization from methanol melted at 66–67°, yield 80%.

Anal. Calcd. for $C_{24}H_{47}N_3O$: C, 73.23; H, 12.03; N, 10.67. Found: C, 74.03; H, 11.80; N, 10.40.

4-Pentadecylsemicarbazone of cyclohexanone after recrystallization from methanol melted at 62–63°.

Anal. Calcd. for $C_{22}H_{43}N_3O$: C, 72.27; H, 11.85; N, 11.49. Found: C, 71.98; H, 10.77; N, 11.47.

2-Methyl-4-heptadecylsemicarbazone of Cyclohexanone.—One gram of the semicarbazide (0.003 mole) and 0.58 g. of cyclohexanone (0.006 mole) in 10 ml. of ethanol containing 3 drops of glacial acetic acid were refluxed for a few minutes and allowed to stand overnight. The alcohol was removed by evaporation and the residue treated with petroleum ether, b.p. 40–60°. The insoluble starting material was removed and the filtrate after evaporation to a small volume yielded after cooling overnight in the ice-chest 250 mg. of the slightly yellow semicarbazone, m.p. 44–48°.

Anal. Calcd. for $C_{25}H_{49}N_3O$: C, 73.62; H, 12.12; N, 10.32. Found: C, 73.24; H, 12.09; N, 10.48.

4-Heptadecylsemicarbazone of cholestenone after recrystallization from ethyl acetate–methanol mixture melted at 78–80°, λ_{max} 273.5 mμ, ϵ_{max} 25,000.

Anal. Calcd. for $C_{45}H_{81}N_3O$: C, 79.46; H, 12.00; N, 6.18. Found: C, 79.10; H, 11.45; N, 6.45.

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DEPARTMENT OF CHEMISTRY
THE MOUNT SINAI HOSPITAL
NEW YORK 29, N. Y.

Alkaloid Biogenesis. IV. The Non-availability of Nicotinic Acid-Carboxyl-C^{14} and its Ethyl Ester for Nicotine Biosynthesis[1]

BY R. F. DAWSON,[2] DAVID R. CHRISTMAN AND R. CHRISTIAN ANDERSON

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For reasons of structural analogy and biological ubiquity, nicotinic acid has been regarded as a possible precursor of the pyridine moiety of the Nicotiana alkaloids.[3] Attempts to secure analytical increases in nicotine content of excised tobacco leaves by feeding solutions of nicotinic acid through cut stems and the transpiration stream have yielded conflicting and inconclusive results.[4] In large part, this is due to the fact, recently demonstrated, that matured excised tobacco leaves do not possess the capacity to synthesize nicotine.[4] The synthetic ability of tobacco roots, on the other hand, has been repeatedly affirmed. This fact, together with the ability of excised tobacco roots to grow indefinitely in sterile culture and to produce nicotine at predictable rates under standard conditions have provided the basis for an isotopic test of the availability of nicotinic acid and its ethyl ester as precursors for nicotine biosynthesis.

Experimental

Nicotinic-C^{14} Acid.—Carboxyl-labeled nicotinic acid was prepared from 3-bromopyridine, *n*-butyllithium and $C^{14}O_2$,

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[1] Research carried out under the auspices of the U. S. Atomic Energy Commission.

[2] Department of Botany, Columbia University, New York 27, N. Y.

[3] E. Winterstein and G. Trier, "Die Alkaloide," 2nd Ed., Borntraeger, Berlin, 1931, pp. 1031.

[4] *Cf.* R. F. Dawson, "Advances in Enzymology," Interscience Publishers, Inc., Vol. 8, 1948, for review.

TABLE I

DATA ON NICOTINE FROM C^{14} FEEDING EXPERIMENTS

	Compound supplied to cultures	1 No. of cultures	2 Length of culture, days	3 Nicotine per culture, mg.	4 Yield of dipicrate, mg.	5 Radioactivity of dipicrate[a] Possible[b]	 Found
A	7.5 mg. nicotinic acid	10	12	0.99	24.3	6.7×10^4	2
B	None	10	12	0.98–1.20	..	....	..
C	15 mg. ethyl nicotinate	15	14	0.82	30	2.2×10^3	8
D	15 mg. ethyl nicotinate	15	15	0.76	22.4	2.2×10^3	6
E	None	15	14–15	0.72–0.90	..	....	..
F	Nicotine dipicrate control		...	....	..	0	4

[a] As c.p.m./cm.^{2} of $BaCO_3$ at infinite thickness. C.p.m./cm.^{2} × 6.16×10^{-3} = mμc./mg. C. [b] If every nicotine molecule had been derived from nicotinic acid or ethyl ester plus an inactive C_5 precursor.

followed by high vacuum sublimation,[5] radioactivity, 3.59, μc./mg. C.

Ethyl-C^{14} Nicotinate.—A mixture of 1.921 g. of nicotinic acid and 0.058 g. of the nicotinic C^{14} acid was suspended in ether. A solution of diazoethane in ether was added slowly, with stirring, until the nicotinic acid was completely dissolved and nitrogen was no longer evolved upon further addition of diazoethane. The ether was removed under reduced pressure and the ester transferred to a small distilling flask, from which it was distilled at less than 0.1 mm. The distillate had a refractive index nearly identical to that of an authentic sample of ethyl nicotinate. It weighed 2.18 g., a yield of 90%. The radioactivity was about 0.1 μc./mg. C.

Root Cultures.—Tobacco roots (*Nicotiana tabacum*, var. Turkish) were grown according to the procedures of White[6] except for the addition to White's medium of traces of copper and molybdenum. Under uniform growth conditions (incubation at 30°, culture fluid volume 33 ml.) each root tip (initially 2–3 cm. long) produced in 5 weeks 20 mg. of dry tissue and 0.5 mg. of nicotine.

Feeding the Radioactive Compounds.—Aqueous solutions of the acid or of its ester were autoclaved and pipetted aseptically into the culture flasks after 12–15 days of root growth. For another 14–16 days, during which growth occurred uninterruptedly, the cultures were incubated in the dark at 30°. At the end of this time, the contents of the flasks were quantitatively removed, the tissues ground in a mortar with sharp sand and 3 ml. of 10% trichloroacetic acid, the combined fluids brought to boiling and filtered. The resulting extract was then continuously extracted with ether from an excess of $Mg(OH)_2$ for 36 hours.

Isolation and Estimation of Nicotine.—Excess ether was removed from the above extracts over *ca.* 20 ml. *N* HCl on a water-bath. The remaining acidic solution of nicotine was made to 25 ml. with *N* HCl, mixed well and optical densities measured at 236, 259.5 and 282 mμ in a Beckman model DU spectrophotometer at 0.5 mm. slit width. Previous experience has shown that the nicotine content of such extracts estimated by the method of Willits, Swain, Connelly and Brice[7] agreed closely with estimates based upon measurements of the optical densities of steam distillates.

For isolation of the alkaloid, the acidic solutions remaining from the above procedure were recombined, made alkaline with NaOH and extracted continuously in a modified Soxhlet apparatus for 12 hours. The ethereal solutions were distilled over aqueous picric acid solution and the resulting samples of nicotine dipicrate were diluted with equal amounts of carrier and recrystallized to appropriate melting point (*ca.* 224°). These picrates were converted to $BaCO_3$ by the wet combustion method of Van Slyke and Folch[8] as modified in this Laboratory.[9] The resulting $BaCO_3$ was collected on disks of standard geometry and counted for 32 minutes in a nucleometer at infinite thickness.[10]

The well-known difficulty of securing complete combustion of nicotine was overcome by using 20–30 minutes of digestion. Careful tests of quantitative recovery of carbon as CO_2 indicated the efficacy of this procedure. In routine operation, however, the narrow margin between complete distillation of CO_2 and carry-over of nitric acid arising from the relatively large quantity of picric acid in the alkaloid dipicrate prevented use of this procedure for obtaining analytical figures for carbon. Because of this difficulty, moreover, distillations of CO_2 in the purification step were so conducted as to be complete for this gas even though some nitric acid was also obtained. The net result was, of course, that many routine analytical figures for carbon were high. In subsequent experiments, the oxalic acid salt of nicotine has been employed with far more satisfactory results.

Results

Table I contains the results of the culture experiments. The negligible activity of the carbonate samples indicates that neither nicotinic acid nor its ethyl ester had been incorporated into nicotine during the culture period. In view of the comparative length of the latter, it seems safe to conclude that nicotinic acid is unavailable directly and that the carboxyl carbon is unavailable indirectly as a source of alkaloid intermediates in the cell.

These results cannot have been complicated by the failure of the roots to absorb nicotinic acid or its ester. Experience in the laboratory of the senior author has shown that nicotinic acid is readily absorbed by excised roots, embryos and leaves of various species of plants including tobacco. Furthermore, Beevers, Goldschmidt and Koffler have recently demonstrated the utility of esters of biologically active, chemically weak acids in overcoming permeability difficulties.[11]

The possibility that nicotinic acid may have been used in a competitive process and hence removed from the sphere of alkaloid biosynthesis was removed by showing that radioactive nicotinic acid could be extracted from the aqueous residue left after the removal of nicotine. These residues were extracted continuously with ether, first in the presence of 2 *N* HCl and later at *p*H 3.0 in order to remove nicotinic acid. The acid was transferred from ether to *N* HCl (25 ml.) and its concentration estimated by suitable measurements in the ultraviolet. Approximately 2 mg. of nicotinic acid was recovered from the cultures which had been supplied with the free acid (row A, Table I). Upon removal of solvents the residue showed an activity of 0.75 μc./mg. C.[12] When the same procedure was applied to the residues from the alkaloid extraction of the ethyl nicotinate experiment, a

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(5) A. Murray, III, W. W. Foreman and W. Langham, THIS JOURNAL, **70**, 1037 (1948).

(6) P. R. White, "A Handbook of Plant Tissue Culture," Cattell Press, Lancaster, Pa., 1943.

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(8) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940).

(9) R. Steele and T. Sfortunato, Brookhaven National Laboratory publication BNL-T-6 (1949).

(10) R. Christian Anderson, Y. Delabarre and Aksel A. Bothner-By, *Anal. Chem.*, **24**, 1298 (1952).

(11) H. Beevers, E. P. Goldschmidt and H. Koffler, *Arch. Biochem.*, **39**, 236 (1952).

(12) These analyses were performed by oxidation of the samples to CO_2 on a standard micro-combustion train, followed by counting the CO_2 in gas counting tubes with a proportional counter. See footnote 9.

strongly absorbing solution was obtained which possessed peaks at 229 and 282 mμ and a minimum at 245 mμ. This material was hydrolyzed with 2 *N* HCl in the autoclave and again extracted with ether in the above manner. *Ca.* 0.5 mg. of active nicotinic acid was obtained; its specific activity was not determined. The nature of the absorbing substance(s) in the first extract at *p*H 3.0 is unknown.

It was noted that the insolubles from the ethyl nicotinate root cultures, after precipitation with trichloroacetic acid at 100°, were radioactive to the extent of 0.008 mμc./mg. C.[12] This fraction normally contains cellulose, protein and other high molecular weight substances.

Inasmuch as nicotinic acid has recently been shown to originate from nicotine during the fermentation of cigar filler tobacco,[13] it thus appears that whatever relationship the two may have in the living plant is confined to the catabolic side of metabolism and is unconcerned with nicotine biosynthesis.

[13] W. G. Frankenburg and A. M. Gottscho, *Ind. Eng. Chem.*, **44**, 301 (1952).

CHEMISTRY DEPARTMENT
BROOKHAVEN NATIONAL LABORATORY
UPTON, LONG ISLAND, N. Y.

N-Nitrosoacetanilide as Polymerization Initiator

BY DELOS F. DETAR[1] AND CONSTANCE S. SAVAT[2]

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It has been shown by Blomquist, Johnson and Sykes[3] and by Hey and Misra[4] that substituted N-nitrosoamides will initiate the polymerization of styrene, methyl methacrylate and acrylonitrile. N-Nitrosoacetanilide itself does not appear to have been tested as an initiator. Although the initiator action suggests that free radicals are being produced as shown in the equation

$$\text{ArN(NO)acyl} \longrightarrow \text{ArN=NOacyl} \longrightarrow \text{Ar.} + \text{N}_2 + \text{acyl}$$

it was found experimentally by both groups of workers that a bromine-containing aryl group gave a bromine-containing polymer but that strangely enough bromine in the acyl group showed much less tendency to become incorporated in the polymer. This result seems to be in marked contrast to the behavior of *p*-bromobenzoyl peroxide[5] with styrene, which leads to incorporation of more bromobenzoate residue than bromophenyl residue.

Since we have been interested in potential sources of free radical intermediates, we have carried out some further polymerization experiments with N-nitrosoacetanilide to see whether this compound might possibly be a cationic initiator (a conceivable explanation of the bromine results) and to get further data about the initiator efficiency. Methyl methacrylate, acrylonitrile and styrene were polymerized, and qualitatively less polymerization occurred in the presence of *sym*-trinitrobenzene. Vinyl acetate and vinylpyridine showed little if any polymerization. Isobutylene was unaffected; there seemed to be no trace even of low molecular weight material such as trimers or tetramers. In six experiments the weight of the residue was zero within the experimental error after subtracting out the weight of the initiator products and of the inhibitor (Table I).

TABLE I

ATTEMPTED POLYMERIZATION OF ISOBUTYLENE

C_4H_8, g.	Nitroso-acetanilide, mmoles	*sym*-Trinitro-benzene, mmoles	Wt. of residue Obsd.	Wt. of residue Expected[a]
7.3	0	0	0	0
6.3	1	0	0.17	0.13
7.4	1	1	.24	.23
6.8	2	0	.33	.26
7.2	2	2	.53	.46
6.9	4	0	.51	.53
6.9	4	4	.93	.93

[a] Allowing for loss of nitrogen from the N-nitrosoacetanilide.

The styrene experiments are reported in Table II. Azo compounds and other products of initiator decomposition made all of the polymers highly colored.

TABLE II

AMOUNT OF POLYSTYRENE FORMED WITH N-NITROSOACETANILIDE AS INITIATOR

87 mmoles (9.2 g., 10 cc.) styrene used in each run

Nitroso-acetanilide, mmoles	*sym*-Trinitro-benzene, mmoles	Wt. polymer + NA residue (+ TNB) I[a]	Wt. polymer + NA residue (+ TNB) II[a]	Net wt. of polymer I[a]	Net wt. of polymer II[a]
0	0	0.00	0.00	0.00	0.00
0.5	0	0.86	0.96	0.79	.89
1	0	1.46	1.14	1.29	.97
2	0	1.55	1.24	1.28	.97
4	0	1.91	1.52	1.38	.99
8	0	2.47	1.93	1.41	.87
0.5	0.25	0.47	0.17	0.34	.04
1	0.5	.64	.34	.40	.10
2	1	.87	.63	.40	.16
4	2	1.42	1.19	.49	.26

[a] I. Tubes allowed to stand at 2–4° in the dark for five days, contents then poured into beakers and allowed to evaporate to constant weight at room temperature and atmospheric pressure. II. Reaction for two days in the dark at room temperature.

The effect of trinitrobenzene and the fact that the acrylates are polymerized readily while styrene is polymerized poorly[6] and isobutylene not at all are consistent with the hypothesis that a free radical rather than a cationic polymerization is taking place.

The low yields of polystyrene may result from such factors as inhibition due to reaction products and to wastage of initiator. But even in the complete absence of these effects only a low yield of polymer is to be expected for such a fast decomposing initiator. For a first order initiation reaction of 100% efficiency and for a bimolecular termination process without inhibition the amount of polymer

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[1] Department of Chemistry, University of South Carolina, Columbia, S. C.

[2] From the M.S. Thesis of Constance S. Savat, 1950.

[3] A. T. Blomquist, J. R. Johnson and H. J. Sykes, THIS JOURNAL, **65**, 2446 (1943).

[4] D. H. Hey and G. S. Misra, *Discussions Faraday Soc.*, **2**, 279 (1947).

[5] P. D. Bartlett and S. G. Cohen, THIS JOURNAL, **65**, 543 (1943).

[6] C. Walling, E. R. Briggs, W. Cummings and F. R. Mayo, *ibid.*, **72**, 48 (1950).