methyl groups, 2 methylenedioxyphenyl groups and a benzoyl moiety.

Reduction of otobanone with $NaBH_4$ in MeOH afforded needle-like crystalline alcohol (II) which showed OH stretching absorption at 3320 cm^{-1}, but no absorption of a conjugated carbonyl group. Compound (II) was treated with *p*-tosylic acid in benzene under reflux, and gave a liquid (III) [ν_{max} 1630 and 810 cm^{-1}, no –OH absorption; λ_{max} 241, 273.5 and 281 nm (log ε 4.14, 4.15 and 4.19)]. Dehydrogenation of compound (III) by dichlorodicyanobenzoquinone produced a phenyl naphthalene derivative (IV) (m.p. 183–185°) which was identified with tetradehydrootobain[5]. Treatment of compound (II) with acetic anhydride in pyridine yielded an acetate (V), m.p. 139.5–140.5, ν_{max} 1720 cm^{-1}, which was treated by reaction with Zn in AcOH under reflux to give unexpected elimination product (III). Hydrogenation of (III) with 5% Pd-C in MeOH gave dihydro compound (VI) (m.p. 136–137°) which was identified as otobain[5]. The evidence concluded that otobanone had to be 1-oxo-otobain as drawn (I) or its C_2 epimer. Further evidence to confirm otobanone to be 1-oxo-otobain is that compound (I) or (V) afford otobain by the reaction of hydrogenolysis with 5% Pd-C.

The assignment of 1-hydroxyl in (II) is in α-quasi-equitorial orientation by the observation that C_1H (with great diquasiaxial coupling constant) exhibited at τ_{CDCl_3} 4.12 (J = 8 Hz) and τ_{CDCl_3} 3.76 (J = 9 Hz) in its acetate (V) and benzoate (VII) derivative, respectively.

Both the configuration and the conformation of ring B in otobanone are the same as that of otobain were defined by the NMR-spectrum. C_4H in otobanone shows great coupling constant at τ_{CDCl_3} 6.28 (J = 9.2 Hz), and the methylenedioxy-protons attached to ring A are markedly different. In otobanone, this is a proton of typical AB system in which the coupling constant is 1.2 Hz and the chemical shift is 0.1 ppm. This is to be expected from formula (I) provided that the conformation of ring B is pseudochair form (VIII), and that in this conformation the phenyl group is in quasi-equatorial. The conformation of ring B in (III) is obviously different from otobanone by the observation of its NMR-spectrum. C_4H in (III) shows less broad singlet at τ_{CDCl_3} 6.00 ($W_{1/2}$ = 3 Hz), and the methylenedioxy-protons attached to ring A exhibit equivalent at τ_{CDCl_3} 4.07 (the same chemical shift with other methylene-protons attached to ring C). This is to be expected from formula (III) provided that the conformation of ring B is an other pseudo-chair form (IX) and that in this conformation the phenyl group is in quasi-axial.

Compound (III) prefers conformer (IX) to conformer (VIII) ascribing to great steric hindrance between ring C and methylenedioxy group attached to ring A in latter conformer. The fact that acetate (V) or benzoate (VII) eliminates spontaneously in $CDCl_3$ solution for 6 days under room temperature to gave (III), is further proof.

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Isolation of the Insect Paralyzing Agent Coniine from *Sarracenia flava*

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Summary. As part of a study to clarify the relationship between insects and the insectivorous plant *Sarracenia flava*, we have observed the presence of the paralyzing agent coniine in the volatile constituents. Fire ants have been used as the test organism in bioassay studies for paralyzing activity.

The pitcher plant, *Sarracenia flava*[4], has long been known to be insectivorous[5]. This characteristic, in conjunction with the conspicuous leaves and flowers, has stimulated interest among scientists in the unique relationship of this plant to the insect population[6]. A variety of insects (ants, wasps, bees, butterflies, moths) are known[5] to enter the pitcher where they are subsequently rendered helpless, digested, and absorbed. Lambert[7] has reported experiments, apparently made on fresh plants of *S. purpurea*, in which a volatile base was present which had the characteristic mouse-like odor of coniine. However, Lambert's attempt to isolate the base from the pitchers (leaves) failed because 'the base was present in so small an amount that only the odor was obtained'.

We have now confirmed the presence of coniine in the volatile fraction of *S. flava*. We have also demonstrated its insect paralyzing activity by administering coniine to fire ants. Fire ants in a small container were paralyzed within 30 sec when exposed to 100 ng of coniine. The insects died upon further exposure.

Isolation procedures. Sarracenia flava leaves were collected 14 miles due east of Panama City, Florida in June

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[4] The plant material used was identified as *Sarracenia flava* (Sarraceniaceae) by Dr. Sidney McDaniel, Department of Botany, Mississippi State University. A voucher (preserved) specimen (SM-16, 702) representing material collected for this investigation is available for inspection at the Herbarium of the Department of Botany, Mississippi State University.

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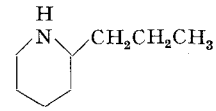
and stored at 0 °C. 45 kg of this plant material was chopped and steam distilled in batches in an all glass system for 3 h. The distillate was extracted with anhydrous ethyl ether which was removed in vacuo to give 8.0 g of are essential oil. The total distillate, which showed paralyzing activity, was placed on a jacketed Florisil column and eluted successively with 100% pentane, 5% ethyl ether in pentane, 15% ethyl ether in pentane, 50% ethyl ether in pentane, and 100% ethyl ether. Bioassay studies showed that physiological activity resided in the fractions eluted with 50% ethyl ether in pentane and 100% ethyl ether.

Separation of the basic components of these two fractions by standard techniques and further chromatography resulted in the isolation of a base (5 mg) with molecular formular $C_8H_{17}N$ (1.9%) from the 100% ethyl ether eluent. The GLC, TLC and mass spectrum of this base from *S. flava* leaves were in complete agreement with an authentic sample of coniine[8,9]. The 5 most intense fragment ion values of the mass spectrum of coniine in order of decreasing relative abundance with the proposed parent ion sixth are at *m/e* 59, 39, 41, 99, 126, 127.



Coniine (2-*n*-propylpiperdine)

Bioassay procedures. The bioassay apparatus consisted of a fibrous soxhlet thimble and a large test tube with a diameter just large enough to accomodate the thimble. The thimble was impregnated with a solution of the fraction to be tested and the solvent (ether or pentane) was allowed to completely evaporate. The thimble was placed in the bottom of the tube and 10 fire ants were placed in the bottom of the thimble. Paralytic activity was indicated when the ants failed to crawl out of the thimble. Thimbles to which only the solvent had been added were used as controls.

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Hepatic and Cerebral Coenzyme A Contents after Intravenous Injection of Coenzyme A in Rats

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Summary. Hepatic CoA concentrations and contents were significantly higher in rats having received i.v. CoA injections than in control rats. Maximum hepatic CoA concentrations were found 0.5–1 h after injection. In rat brain, no increase in CoA concentration was detected after i. v. injection of CoA.

In a previous paper[3] we have shown that addition of Coenzyme A (CoA) and some of its precursors led to an increase in the O_2-consumption of rat liver slices. The CoA concentration in the slices, however, only increased after addition of CoA itself or of dephospho-CoA (DPCoA). In this paper, the results of experiments on in vivo uptake of i.v. administered CoA by rat liver and brain are presented. In addition, we report our observations on the rise in CoA concentration in rat liver upon starvation.

Materials and methods. CoA (containing 80–85% CoASH), NAD, and the enzymes required for CoA determination were purchased from Boehringer, Mannheim.

Female white rats of 170–250 g body weight were used. Starved rats were fasted for 16 h with no restriction on water intake. The CoA injections were given into the tail vein. Each rat received 10 µmoles CoA dissolved in 0.25 ml 5% glucose in water and neutralized to a pH of 6–7 with $NaHCO_3$. Control animals received 0.05 ml isotonic saline +0.2 ml 5% glucose solution.

CoA determination were performed as previously[3], using the kinetic method with phosphotransacetylase described by MICHAL et al.[4]. Livers and brains were homogenized immediately after decapitation in 2 volumes of water at 0–4 °C. Dry weights were determined in the homogenates. Prior to measurement of CoA, the homogenates were deproteinized with perchloric acid at a final concentration of 0.4 *M*, and neutralized with K_2CO_3. In the resulting extracts the acid soluble fraction of CoA, i.e. the sum of CoASH, acetyl-CoA and CoA disulfide, was determined, the latter having been reduced by means of a preincubation with dithioerythritol.

To determine concentrations of total CoA, acid insoluble acyl-CoA was hydrolyzed prior to deproteinization by an incubation with 0.8 *M* NH_3 and 0.02 *M* mercaptoethanol at room temperature. Direct determinations of acyl-CoA in the washed precipitates from the perchloric acid extraction resulted in concentrations, which corresponded fairly well to the difference between total CoA and acid soluble CoA. The CoA tissue concentrations were not corrected for the CoA content in blood as CoA determinations revealed that the CoA blood concentrations $^1/_2$ h after injection were of the same magnitude as in untreated animals, namely 0.2–0.7 nmoles/ml.

Results. The effect of starvation on concentration and content of CoA in rat liver. Our experiments on uptake of CoA by rat liver slices[5] had revealed that CoA concentrations were sonsiderably higher in livers from starved than in livers from fed rats. A similar rise in heptatic CoA concentration following starvation has been observed by other authors[6–8].

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