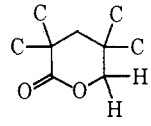
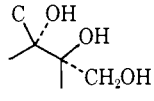
group (δ 1.24, s) in addition to that present originally (δ 1.09, s). Reduction also confirmed the presence of a δ-lactone in nepetaefuran (ν_{max}^{Nujol} 1730 cm^{-1}), a functionality which had been indicated previously by formation of a potassium salt of **2** upon vigorous basic hydrolysis. Acidification of this salt induced immediate relactonization. Part structure A follows



A

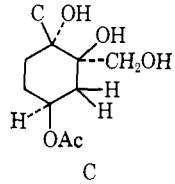
from the observation that the carboxylate cannot be methylated and that the reduction product, **4**, contains two AB quartets at δ 3.27 and 4.19 (J = 11 Hz) and δ 3.88 and 4.29 (J = 13 Hz).

Further clarification of the structures of these diterpenoids became possible with the discovery of a third substance, nepetaefuranol (**5**), $C_{22}H_{30}O_8$, mp 253–255°, $[\alpha]^{25}D$ +17.2° (c 1.05, MeOH), in the extract from *L. nepetaefolia*. Nepetaefuranol contained a primary alcohol (δ 3.30 and 3.79, 2 H, AB quartet, J = 11 Hz) and furnished a monoacetate, mp 185–186°, containing two hydroxyl groups. Its relationship with **2** was established by treatment of the latter with perchloric acid, which gave **5**; the reverse transformation was accomplished by treatment of the tosylate of **5** with base. Nepetaefuranol therefore contains the part structure represented as B. Oxidation of **5** with sodium meta-



B

periodate yielded a norketone, $C_{21}H_{22}O_7$, mp 188–189°, ν_{max}^{Nujol} 1720–1740 cm^{-1} (broad), from which acetic acid was eliminated on alumina to give an α,β-unsaturated cyclohexenone, mp 175–177°, m/e 330, ν_{max}^{Nujol} 1670 cm^{-1}, δ 6.41 (1 H, d, J = 10 Hz) and 6.81 (1 H, d, J = 10 Hz). This allows extension of part structure B to C.



C

Structural features uncovered in the course of degradative work suggest that **1**, **2**, and **5** are diterpenes of the labdane type,[5] and chemical data as well as biogenetic theory are accommodated uniquely in these formulas. The position and orientation of the lactone are dictated by the conspicuous AB pattern of the lactone methylene protons in **5** (δ 4.03 and 5.88, J = 12 Hz) and degradation products containing an 8-β-OH function, where a pronounced downfield shift occurs due to a 1,3-diaxial relationship.[6] Unambiguous proof of the structure of nepetaefuran, and hence of nepetaefolin and nepetaefuranol, was obtained by correlation with leonotin (**6**) of known structure and relative stereochemistry.[7] Treatment of **4** with tosyl chloride in pyridine gave a mixture of the two primary monotosylates which, upon hydrogenolysis with $LiAlH_4$ in THF, afforded leonotol (**7**), mp 136–138°, probably by way of an intermediate cyclic ether.[8] Leonotol had previously been acquired by reduction of **6** and the latter, in turn, has been correlated with marrubiin.[9] The relative stereochemistry at all six chiral centers of **2** and **5** are thereby defined, as are the corresponding centers in nepetaefolin (**1**), but configuration at the additional spirocarbon of the latter remains unspecified.

The biogenetic implication that **1** is the immediate precursor of **2** in *L. nepetaefolia* is upheld by isolation studies to be reported subsequently, but it may be noted that observations in parallel with ours have recently been made in the marrubiin series.[10] Details of the biosynthetic processes leading from the isoprenoid skeleton to the spirodihydrofuran system, however, are as yet obscure.

Acknowledgments. We are grateful to Mr. M. Hasmathullah, Warrenville, Trinidad, for a supply of *Leonotis nepetaefolia*. Generous financial support was provided by the National Science Foundation (Grant No. GP-15,331) and by Hoffmann-LaRoche, Inc.

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Incorporation of 1,3-Dimethyl-1-pyrrolinium Chloride in *Nicotiana glutinosa*. Biosynthesis of a Substituted Nicotine[1]

Sir:

The study of the biosynthesis of natural products in plants has been carried out almost exclusively by means of precursor feeding experiments, although the importance of alternate methods such as short-term biosynthesis with $^{14}CO_2$ has been stressed.[2] Ideally, only the natural precursor should be incorporated efficiently into the natural product; however, incorporation of an unnatural precursor into a natural product is well documented.[3] Although theoretically possible, neither the incorporation of a natural precursor into an unnatural product[4] nor the incorporation

(1) This investigation was supported in part by Grant No. MH 12797 from the National Institute of Mental Health, U. S. Public Health Service, and the U. S. Atomic Energy Commission. Dedicated to Professor Kurt Mothes on the occasion of his 70th birthday.

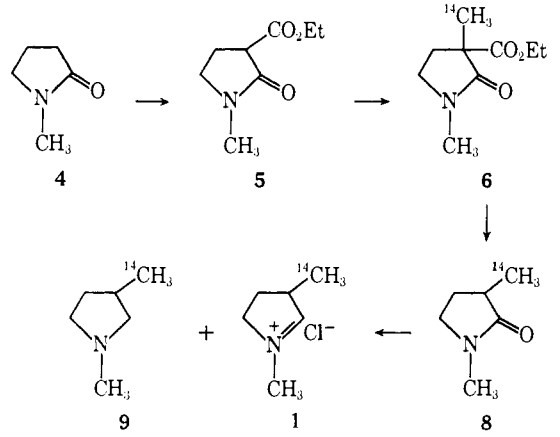
(2) A. A. Liebman, B. P. Mundy, and H. Rapoport, *J. Amer. Chem. Soc.*, **89**, 664 (1967).

(3) G. Blaschke, H. I. Parker, and H. Rapoport, *ibid.*, **89**, 1540 (1967); T. J. Gilbertson and E. Leete, *ibid.*, **89**, 7085 (1967).

(4) Preliminary results indicate that examples of this type have been observed in studies on nicotine and morphine metabolism.

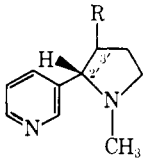
of an unnatural precursor into an unnatural product, closely related to the natural one, has been previously reported. We now provide an example of biosynthesis involving the latter type of precursor incorporation.

Since 1-methyl-1-pyrrolinium chloride (**2**) has been found to be an efficient precursor of the pyrrolidine ring of nicotine (**3**),[5] 1,3-dimethyl-1-pyrrolinium-3-$^{14}CH_3$ chloride (**1**) was selected as the candidate unnatural precursor and synthesized as shown.[6] 1-Methyl-2-pyrrolidone (**4**), condensed with diethyl carbonate with sodium hydride as base, gave ester **5**. 1,3-Dimethyl-3-carbethoxy-2-pyrrolidone-3-$^{14}CH_3$ (**6**)[7] was obtained by alkylating the sodium enolate of **5** with methyl-^{14}C iodide.[8] Hydrolysis of the alkylated ester **6** (specific activity 2.71×10^7 dpm/mmol) quantitatively



gave acid **7** (specific activity 2.68×10^7 dpm/mmol) which on decarboxylation at 150–160° gave 1,3-dimethyl-2-pyrrolidinone (**8**).[9] Reduction of **8** with lithium aluminum hydride gave in 92% yield a mixture of pyrrolinium salt **1** (70%) and pyrrolidine **9** (30%). Chromatographies on silica gel, eluting with ethanol–0.1 *N* HCl (2:1), followed by ion exchange gave pure **1** in 40% overall yield from **5**.

In order to examine the possibility of biosynthesizing 3′-methylnicotine (**10**), **1** was administered in portions over a period of several days to an aerated hydroponic solution[10] containing four *N. glutinosa* plants in each



3, R = H
10, R = CH_3

experiment (Table I). At the end of the biosynthetic experiment, the alkaloidal fraction was isolated as described[11] and fractionated by preparative gas liquid partition chromatography.[12] In addition to the normal *Nicotiana* alkaloids,[11] a peak at retention time 31.2 min was also present in a yield of 5–11% (Table I).

Table I. Administration of 1,3-Dimethyl-1-pyrrolinium-3-$^{14}CH_3$ Chloride (**1**) to *Nicotiana glutinosa*[a] and Incorporation into 3′-Methylnicotine (**10**)

Expt	Pyrrolinium salt **1** fed mg	Pyrrolinium salt **1** fed dpm	Incorpn into 3′-methylnicotine (**10**) dpm	Incorpn into 3′-methylnicotine (**10**) %
1	40.4[b]	8.10×10^6	4.22×10^5	5.2
2	200.4[c]	3.97×10^7	4.34×10^6	10.9[d]

[a] For the preparation of the plants, see ref 11. [b] Administered over a 5-day period followed by 1 day of growth. Total weight of the four plants was 261 g; their age was 66 days. [c] Administered in increasing amounts over a period of 8 days to 59-day-old plants. Total weight of the four plants was 54 and 139 g at the start and finish, respectively. [d] Using nicotine (**3**) as the standard, glpc analysis indicated the presence of 56.0 mg of nicotine (**3**) and 21.6 mg (8.3%) of 3′-methylnicotine (**10**).

The new substance has been characterized as 3′-methylnicotine (**10**). Its ultraviolet spectrum shows a $\lambda_{max}^{C_2H_5OH}$ of 261 nm and is identical in all respects with that of nicotine.[13] The specific activity of **10** was determined by a combination of uv absorption and liquid scintillation counting to be 2.76×10^7 dpm/mmol, in excellent agreement with its precursor **1**. The mass spectrum of **10** gave a molecular ion at *m/e* 176 (22% of base) along with peaks at *m/e* 175 (7), 133 (100), 119 (6), and 98 (53), all analogous with those of nicotine.[14] High-resolution mass spectroscopy established the molecular formula as $C_{11}H_{16}N_2$ for *m/e* 176 (calcd: 176.1313; found: 176.1323) and $C_6H_{12}N$ for *m/e* 98 (calcd: 98.0970; found: 98.0974) for the 1,3-dimethyl-1-pyrrolinium fragment formed by α cleavage. The nmr (CCl_4) shows peaks at δ 8.40 (m, 2 H), 7.60 (m, 1 H), 7.17 (m, 1 H), 3.20 (m, 1 H), 1.4–2.6 (m, 5 H), 2.10 (s, 3 H, N-C*H*$_3$), and 0.97 (d, 3 H, >CHC*H*$_3$) consistent with structure **10**.

Biogenetically, 3′-methylnicotine (**10**) would be expected to have the same absolute configuration at the 2′-carbon as nicotine (**3**) which has been assigned the *S* configuration with reference to L-proline,[15] L-serine,[16] and optical rotary dispersion measurements.[17] The CD curve of **10** (in 95% EtOH) gave a molecular ellipticity [Θ] at 260 nm of +22,800 (peak); **3** showed a $[\Theta]_{271}$ −7090 (trough) in addition to $[\Theta]_{261}$ +24,800 (peak). Although **3** showed a weaker negative Cotton effect at 273 nm in the ORD,[17] this absorption was absent in both the CD and ORD of **10** due possibly to the presence of an adjacent asymmetric center. As a consequence, **10** is tentatively assigned the *S* configuration at the 2′ carbon; the presence of the alkyl methyl in **10** as a single doublet in the nmr indicates that only one of the possible diastereomers was formed biosynthetically.

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(12) Chromatography was on a 15 ft × 1/4 in. column of 10% KOH, 10% polybutyleneglycol on 60–80 firebrick, column temperature 176°, flow rate of 90 ml/min. The retention times of nicotine and nornicotine were 28.2 and 47.5 min, respectively.

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The biosynthesis of 3′-methylnicotine (**10**) from 1,3-dimethyl-1-pyrrolinium salt **1** demonstrates that the enzyme system which catalyzes the biosynthesis of nicotine from 1-methyl-1-pyrrolinium salt **2** and a nicotinic acid derivative is not completely specific, and its requirements may become definable through experiments such as these. In addition the formation of unnatural products from unnatural precursors *in vivo* should be useful in the preparation of analogs of biologically active natural products (with high specific activity if desired) and in the study of metabolism and interrelationships among alkaloids.

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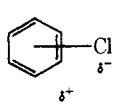
Some Highly Selective Radicals. The Chlorovinylation of Hexane

Sir:

Early investigations of product distributions following chlorinations, brominations, or oxidations of linear paraffin hydrocarbons indicated a nearly equal reactivity of all methylene hydrogen atoms. However, Asinger and Fell[1] and Fell and Kung[2] have demonstrated that a number of free radicals attack the methylene groups of a linear hydrocarbon in a significantly selective manner. Russell[3] made the important finding that the selectivity of chlorine atoms in the chlorination of dimethylbutane could be greatly accentuated by a solvent such as benzene or carbon disulfide. Fell and Kung chlorinated *n*-heptane and *n*-octane in these solvents and showed that in these systems the methylene hydrogens were far from equivalent in reactivity. The rationale for the remarkable effect of benzene, for example, presupposes a polarized reaction intermediate or loose complex between the chlorine atom and the benzene solvent. Whatever the formalized description of this intermediate, it seems probable that this same



radical is the first step in the addition of chlorine to benzene. If this is so, then highly selective radicals should be derivable from molecules already containing bound chlorine. Just such highly selective radicals are indicated to be intermediates in the production of chlorovinyl derivatives by the peroxide-catalyzed combination of polychloroethylenes and paraffin hydrocarbons discovered by Schmerling and West.[4] Unfortunately, these authors did not have the benefit of gas chromatography; otherwise it is doubtful that they would have hypothesized a chlorine atom intermediate as the reaction chain carrier because the isomer distributions in no way correspond to those that would be predicted from chlorine atom attack. We have treated *cis*-1,2-dichloroethylene, trichloroethylene, and tetrachloroethylene with hexane (1:6 *M* ratio) in sealed Pyrex bomb tubes at 125° with di-*tert*-butyl peroxide (1–2 mol %) as the reaction initiator (reaction times, *ca.* 16 hr). The product isomers were separated by glc and the reactivities of the hydrogen atoms in the various systems are compared with the reactivities of those same hydrogen atoms toward chlorine (photochlorination of hexane at 65°). The results are summarized in Table I.

The Schmerling–West mechanism as modified (for the case of trichloroethylene–hexane) then becomes

$$\mathrm{ROOR} \longrightarrow 2\mathrm{RO}\cdot$$

$$\mathrm{RO}\cdot + \mathrm{C_6H_{14}} \longrightarrow \mathrm{ROH} + \mathrm{C_6H_{13}}\cdot$$

$$\mathrm{C_6H_{13}}\cdot + \mathrm{CHCl{=}CCl_2} \longrightarrow \mathrm{C_6H_{13}CHCl{-}\dot{C}Cl_2} \not\longrightarrow \mathrm{C_6H_{13}CH{=}CCl_2} + \mathrm{Cl}\cdot$$

$$\mathrm{C_6H_{13}CHCl{-}\dot{C}Cl_2} + \mathrm{C_6H_{14}} \longrightarrow \mathrm{C_6H_{13}CH{=}CCl_2} + \mathrm{HCl} + \mathrm{C_6H_{13}}\cdot$$

where $\mathrm{C_6H_{13}}\cdot$ = $\mathrm{CH_3\dot{C}HCH_2CH_2CH_2CH_3}$ or $\mathrm{CH_3CH_2\text{-}\dot{C}HCH_2CH_2CH_3}$.

Table I. Relative Reactivities of the Hydrogens in Hexane

Hexane	*hv*, 65° Cl_2–hexane	*cis*-CHCl=CHCl + hexane	CHCl=CCl_2 + hexane	CCl_2=CCl_2 + hexane	CHCl=CC_2 + CCl_4 + hexane	CCl_4 + hexane
CH_3	0.35	0.06	0.04	Trace	<0.03	Trace
CH_2	1.15	1.97[a]	1.68	4.24	1.19	1.63
CH_2	1.0	1.0	1.0	1.0	1.0	1.0

[a] The product contains two cis and two trans isomers. The two cis isomers did not resolve on glc. Therefore, the value 1.97 is the ratio of *trans*-$C_4H_9CHCH_3CH{=}CHCl$ to *trans*-$C_3H_7CHC_2H_5CH{=}CHCl$. The ratio of total cis to total trans was 1.44.

Most noteworthy is the unusual selectivity of the chain-carrying radical from tetrachloroethylene and hexane. Over 80% of the reaction is at the 2 position.

A system which possesses its own unique pattern of selectivity and at the same time produces chlorohexane instead of vinylic chloro derivatives is a mixture of carbon tetrachloride, trichloroethylene, and hexane. In this case the reaction system proposed is

$$\mathrm{ROOR} \longrightarrow 2\mathrm{RO}\cdot$$

$$\mathrm{RO}\cdot + \mathrm{C_6H_{14}} \longrightarrow \mathrm{ROH} + \mathrm{C_6H_{13}}\cdot$$

$$\mathrm{C_6H_{13}}\cdot + \mathrm{CCl_4} \longrightarrow \mathrm{C_6H_{13}Cl} + \mathrm{\dot{C}Cl_3}$$

$$\mathrm{CCl_3}\cdot + \mathrm{CHCl{=}CCl_2} \longrightarrow \mathrm{CCl_3CHCl\dot{C}Cl_2}$$

$$\mathrm{CCl_3CHCl\dot{C}Cl_2} + \mathrm{C_6H_{14}} \longrightarrow \mathrm{CCl_3CH{=}CCl_2} + \mathrm{C_6H_{13}}\cdot + \mathrm{HCl}$$

Referring to Table I where the results of this reaction can be compared with halogenation of hexane by chlorine and by carbon tetrachloride, it seems obvious from

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