CO_2CH_2–), 4.68 (s, 1 H, C-5), 6.10 (dd, 1 H, $J_{7,8}$ = 10 Hz, $J_{7,14}$ = 3 Hz, C-7 H), 6.67 (m, 3 H, C-1, C-2, C-8 H), 7.06 (m, 1 H, C-3 H); IR (Nujol) 1685 (both >CO) cm^{-1}. Anal. ($C_{19}H_{19}NO_4$) C, H, N.

3-Deoxymorphine (13). A solution of 3.10 g (9.54 mmol) of 12 in 30 mL of dry THF was added dropwise, with stirring, to a slurry of 3.63 g (95.4 mmol) of $LiAlH_4$ in 90 mL of THF under argon. After heating the solution at reflux for 24 h, the reaction was chilled in ice and quenched by the careful addition of 3.60 mL of H_2O, 3.60 mL of 15% NaOH, and 10.8 mL of H_2O. The solid which separated was removed by filtration and washed well with THF, and the combined filtrate and washings were evaporated. Column chromatography (silica gel, $CHCl_3$–MeOH–NH_4OH, 89:10:1/$CHCl_3$, 60:40) followed by recrystallization (EtOH) provided 1.54 g (60%) of 13 as white crystals: mp 227.5–229 °C; MS M^+ 269; $[\alpha]^{21}_D$ –218° (c 1.00, MeOH); NMR ($CDCl_3$) δ 2.45 (s, 3 H, NCH_3), 4.16 (br s, 1 H, C-6 H), 4.83 (d, 1 H, J = 7 Hz, C-5 H), 5.28 (m, 1 H, C-8 H), 5.66 (d, 1 H, J = 8 Hz, C-7 H), 6.57 (m, 2 H, C-1, C-2 H), 6.98 (m, 1 H, C-3 H); IR (Nujol) 3555 cm^{-1} (OH). Anal. ($C_{17}H_{19}NO_2$) C, H, N.

The hydrochloride of 13 had mp 269–273 °C dec. Anal. ($C_{17}H_{20}ClNO_2$) C, H, N, Cl.

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Metabolic Oxidation of Nicotine to Chemically Reactive Intermediates

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Studies on the metabolism of nicotine by rabbit liver microsomal fractions in the presence of 0.01 M sodium cyanide have led to the characterization of two isomeric cyanonicotine compounds. The locations of the cyano groups were established by GC–EIMS analyses of the deuterium-labeled products obtained from the specifically deuterium-labeled substrates (*S*)-nicotine-5′,5′-d_2, (*R*,*S*)-nicotine-2′,5′,5′-d_3, and (*R*,*S*)-nicotine-*N*-methyl-d_3. One cyano adduct was shown to be 5′-cyanonicotine, a product previously isolated from similar microsomal preparations. The second cyano adduct was shown to be *N*-(cyanomethyl)nornicotine; this structure assignment was confirmed by synthesis. Formation of *N*-(cyanomethyl)nornicotine appears to occur, at least in part, without prior nitrogen–carbon bond cleavage, implicating the in situ generation of the *N*-methyleniminium species during the course of metabolic oxidative N-demethylation of nicotine.

Many of the biotransformation processes observed in the metabolism of xenobiotics are mediated by hepatic mixed-function oxidases with cytochrome P-450 as the terminal oxidase. Metabolic oxidations of aliphatic amines often occur at carbon atoms bonded to nitrogen, presumably with the initial formation of chemically unstable carbinolamines which may undergo spontaneous carbon–nitrogen bond cleavage to the corresponding dealkylated amines and aldehydes or ketones.[2] The resulting aldehydes may then suffer a further two-electron oxidation to yield carboxylic acids, or in the case of cyclic amines, lactams.[3] According to the proposed pathway, the three unique carbon atoms of nicotine (1) bonded to the pyrrolidine nitrogen atom should form the carbinolamines **2**–**4** as the initial metabolic products (Scheme I). Cotinine (**5**) and nornicotine (**6**), major and minor mammalian metabolites of nicotine,[4] presumably arise via the carbinolamines **2** and **3**, respectively. To date there have been no reports of mammalian metabolites arising from initial hydroxylation at the 2′ position of nicotine.

Evidence for the intermediacy of 5′-hydroxynicotine (**2**) in the biotransformation of 1 to **5** has been reported. In 1960, Hucker et al.[5] showed that rabbit liver microsomal preparations convert 1 to **5** and proposed the carbinolamine **2** as an intermediate which is further metabolized by a soluble aldehyde oxidase to **5**. By blocking the aldehyde oxidase system with cyanide ion, these workers claimed evidence of an equilibrium mixture of **2** and the open-chain amino aldehyde **7** which could be trapped as its 2,4-DNP derivative.

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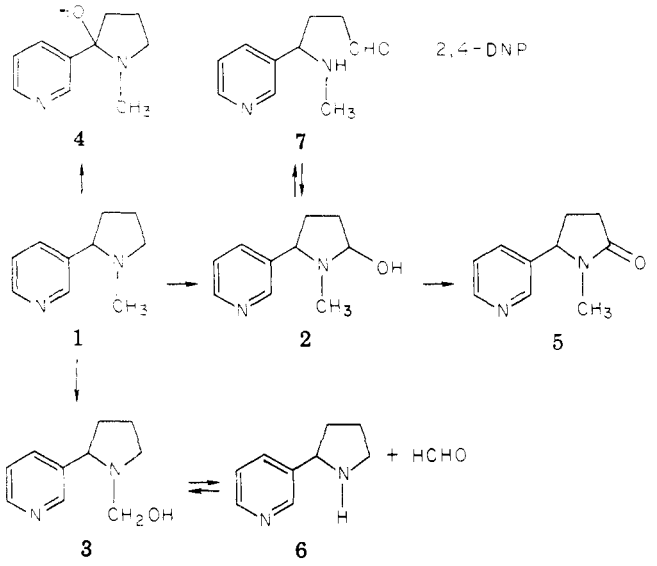
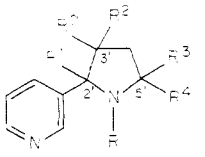
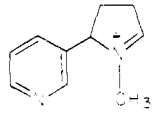
Scheme I. Proposed Pathways for the Formation of Cotinine (5) and Nornicotine (6), the Principal Mammalian Metabolites of Nicotine (1)



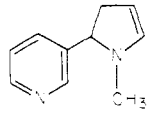
In 1973, Murphy[6a] repeated Hucker's work and reported evidence for the presence of a cyanonicotine derivative in the incubation mixture. He postulated that the initial metabolic hydroxylation product **2** ionizes to the electron-deficient nicotin-$\Delta^{1'(5')}$-iminium ion (**8**), which is



	R	R^1	R^2	R^3	R^4
1	CH_3	H	H	H	H
1-5',5'-d_2	CH_3	H	H	D	D
1-2',5',5'-d_3	CH_3	D	H	D	D
1-NCD_3	CD_3	H	H	H	H
6	H	H	H	H	H
6-2',3',3'-d_3	H	D	D	H	H
9	CH_3	H	H	H	CN
11	CH_3	CN	H	H	H
12	CH_2CN	H	H	H	H
12-NCD_2-	CD_2CN	H	H	H	H
12-2',3',3'-d_3	CH_2CN	D	D	H	H
12-d_5	CD_2CN	D	D	H	H
14	$CH(CN)CH_3$	H	H	H	H



8

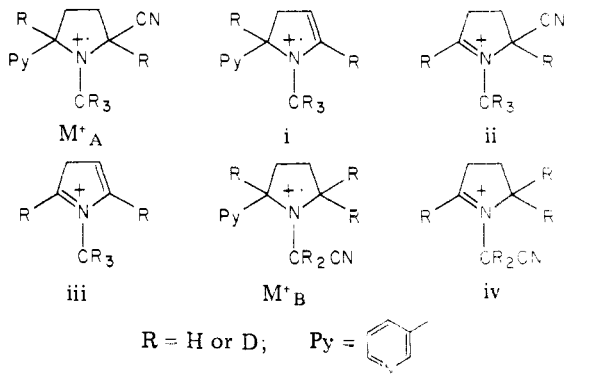


10

trapped by cyanide ion to form the stable adduct 5'-cyanonicotine (**9**). The partial structure of **9**[6b] was established by spectral evidence and by synthesis via mercuric acetate oxidation of nicotine in the presence of cyanide ion. Confirmation of this structure assignment was obtained by an independent synthesis involving a two-electron reduction of cotinine and in situ trapping of the resulting iminium species **8** with cyanide ion.[7]

As part of our studies on the mechanisms of oxidative metabolism of nitrogen-containing compounds,[8] we sought to evaluate further the formation of electrophilic iminium species with the aid of various specifically deuterated nicotine analogues and rabbit liver preparations under incubation conditions similar to those used by Murphy.

Table I. EIMS of the Two Isomeric Cyanonicotine Compounds Obtained from Incubations of Nicotine and Deuterated Nicotine Analogues



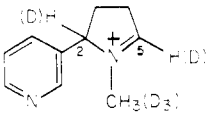
	A				B	
substrate	M^+	i	ii	iii	M^+	iv
1	187	160	109	82	187	109
1-5',5'-d_2	188	161	110	83	189	111
1-2',5',5'-d_3	189	162	111	84	190	112
1-NCD_3	190	163	112	85	189	111

When unlabeled nicotine (1) was incubated with a 100000*g* microsomal preparation in the presence of the appropriate cofactors (NADPH, $MgCl_2$) and sodium cyanide, GC analysis of the base extract showed, in addition to nicotine, two major isomeric products, A and B (10.2 and 13.6 min, respectively), which gave the mass spectra summarized in Table I. The GC–EIMS of neither compound A [M^+ 187 (23.7%), 160 (65%), 109 (100%), and 82 (82.5%)] nor compound B [M^+ 187 (35%) and *m/e* 109 (100%)] corresponded to the spectrum reported by Murphy for compound **9** [M^+ 187 (2%), 160 (45.3%), 109 (24%) and 82 (100%)]. The GC–EIMS analysis at a higher separator temperature did not alter the spectrum of B. The EIMS of peak A at the higher separator temperature, however, was identical to the spectrum reported by Murphy for 5'-cyanonicotine. These temperature-dependent differences in the mass spectra of **9** can be explained in terms of thermal elimination of HCN from **9** to form the enamine **10** prior to entering the ion chamber.

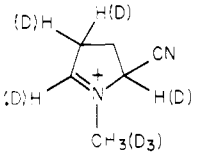
Neither compound A nor B was formed in the absence of cyanide ion. Furthermore, only unmetabolized nicotine was detected in the base extract of incubations employing denatured microsomes (preheated to 100 °C for 10 min) or NADPH-free preparations. These control experiments strongly implicate the obligatory role of the microsomal mixed-function oxidase system in the formation of intermediate species, which then react spontaneously with cyanide ion to generate A and B.

The position of the cyano group of the new cyanonicotine derivative B was unambiguously established with the aid of the specifically deuterated nicotine analogues nicotine-5',5'-d_2 (1-d_2), nicotine-2',5',5'-d_3 (1-d_3), and nicotine-*N*-methyl-d_3 (1-NCD_3). The mass spectral data of A and B isolated from incubates of nicotine and the above deuterated analogues are summarized in Table I. In addition to the parent ion M^+ at 187, the GC–EIMS spectrum of A obtained from 1 shows major peaks at *m/e* 160, 109, and 82 corresponding to ions i, ii, and iii, respectively. The nominal masses of the parent and fragment ions of the various deuterium labeled A are consistent only with the established 5'-cyano structure **9**.

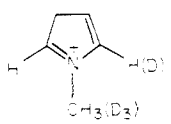
The GC–EIMS of compound B isolated from incubates of 1 shows, in addition to the parent ion M^+ 187, a single fragment ion at *m/e* 109 corresponding to the loss of the



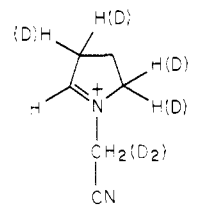
	m/e
i	160
i-5′-d_1	161
i-2′,5′-d_2	162
i-NCD$_3$	163



	m/e
ii	109
ii-5′-d_1	110
ii-2′,5′-d_2	111
ii-NCD$_3$	112
ii-2′,3′,3′-d_3	112



	m/e
iii	82
iii-5′-d_1	83
iii-2′,5′-d_2	84
iii-NCD$_3$	85

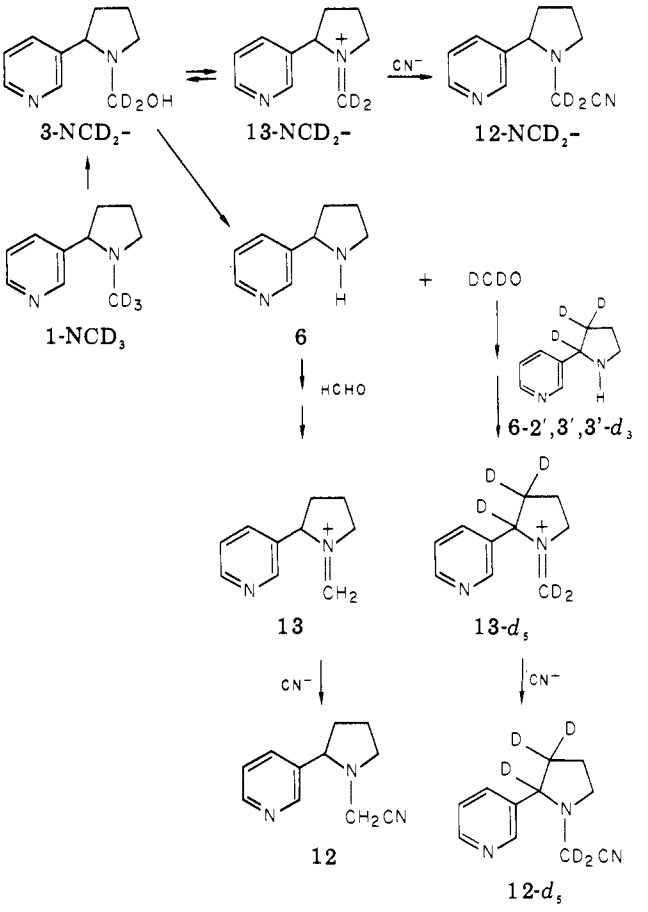


	m/e
iv	109
iv-5′,5′-d_2	111
iv-2′,5′,5′-d_3	112
iv-2′,3′,3′-d_3	112
iv-NCD$_3$	111
iv-d_5	114

pyridyl moiety. With substrates 1-d_2 and 1-d_3, the M^+ and M^+ – pyridine ions of B show retention of all deuterium atoms, thus ruling out as possible structures the 5′-cyano epimer of **9** and the 2′-cyano isomer **11**. On the other hand, compound B formed from the incubation of nicotine-*N*-*methyl*-d_3 gave a parent ion at M^+ 189 and fragment ion, M^+ – pyridine, at *m/e* 111. The spectrum establishes that one of the deuterons of 1-NCD$_3$ has been replaced with a cyano group and, therefore, that the structure of B is *N*-(cyanomethyl)nornicotine (**12**). The major EIMS fragment ion of **12**, therefore, is iv. Although the direct displacement of the hydroxyl functionality cannot be ruled out, the *N*-(cyanomethyl) product presumably results from initial mixed-function oxidation of nicotine to *N*-(hydroxymethyl)nornicotine (**3**) followed by ionization to the nicotine-*N*-methyleniminium ion **13**, which then is attacked by cyanide ion to form the observed product (Scheme II). The absence of a fragment ion resulting from the loss of HCN in the EIMS of **12** is consistent with the lack of a β proton.

The structure of **12** was confirmed by synthesis from nornicotine, formaldehyde, and cyanide ion.[1] With nornicotine as the limiting reactant, the reaction proceeded in good yield at room temperature. The mild reaction conditions required indicated that metabolically formed nornicotine could undergo spontaneous cyanomethylation under our incubation conditions. This suspicion was reenforced by evidence from control experiments. Continuous mixing for several hours of an aqueous solution of nornicotine and sodium cyanide with dichloromethane (presumably contaminated with formaldehyde) followed by GC–EIMS analysis of the extract demonstrated detectable quantities of **12**. Consequently, diethyl ether was employed as the extracting solvent in these experiments. With "aged" anesthetic grade diethyl ether from cans exposed to air and moisture, the presence of small amounts of acetaldehyde led to detectable levels of *N*-(1-cyanoethyl)nornicotine (**14**) in a diethyl ether extract of an aqueous solution of nornicotine and cyanide ion. Nelson et al. have reported similar complications with diethyl ether and dichloromethane in studies concerned with the formation of reactive intermediates during the metabolic

Scheme II. Studies on the Formation of *N*-(Cyanomethyl)nornicotine. Evidence Suggesting the Intermediary Role of the Methyleniminium Ion 13

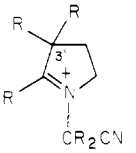


oxidative N-deethylation of lidocain.[9]

Although the control experiments demonstrated that **12** is formed from the metabolism of nicotine in the presence of cyanide ion, there still remained some ambiguity as to the pathway of its formation. As illustrated with nicotine-*N*-*methyl*-d_3 (1-NCD$_3$) in Scheme II, there are at least two routes for the formation of 12-NCD$_2$–. One is the direct route where the metabolic intermediate, presumably the carbinolamine 3-NCD$_2$–, undergoes spontaneous conversion to the electrophilic methyleniminium ion 13-NCD$_2$– without prior N–C bond cleavage. Rapid reaction of 13-NCD$_2$– with cyanide ion would then generate 12-NCD$_2$–. A second, indirect pathway would lead to 12-NCD$_2$– via an initial condensation of metabolically formed nornicotine and formaldehyde. The direct pathway differs from the indirect pathway in that the latter involves cleavage of the N–C bond originally present in the nicotine molecule.

In order to determine whether the direct, indirect, or both pathways operate in the formation of 12-NCD$_2$, a tenfold molar excess of unlabeled formaldehyde (HCHO) was coincubated with nicotine-*N*-*methyl*-d_3. The added HCHO was expected to dilute any metabolically formed DCDO at least 50-fold. Formation of negligible amounts of *N*-(cyanomethyl)nornicotine (**12**) compared to the amount of *N*-(cyanomethyl-d_2)nornicotine (12-NCD$_2$–) would argue for the predominance of the direct pathway to **12**. GC–EIMS base peak analysis of the cyanomethyl compound obtained from this experiment revealed a 1:1 mixture of *N*-(cyanomethyl)- and *N*-(cyanomethyl-d_2)-nornicotine (*m/e* 109 vs. 111). Thus, to a significant extent, **12** appears to be formed without prior N–C bond

Table II. Selected Ion-Current Record of the *N*-(Cyanomethyl)nornicotine GC Peak from the Incubation of Nicotine-*N*-*methyl*-d_3 in the Presence of Cyanide Ion; (A) Added HCHO and Nornicotine-2′,3′,3′-d_3 Added at the End of the Incubation; and (B) Equal Molar Nornicotine-2′,3′,3′-d_3 as Cosubstrate



R = H or D

		ratio %	
m/e	ion	A	B
109	iv	33	5
111	iv-NCD_2–	30	100
112	iv-2′,3′,3′-d_3	100	39[a]
114	iv-d_5	5	53

[a] The ion current seen at *m/e* 112 in this experiment is largely due to ion ii-NCD_3 of 5′-cyanonicotine-*N*-*methyl*-d_3.

cleavage, suggesting that the reactive methyleniminium ion **13** is generated, at least in part, by the direct pathway in the course of the in vitro metabolism of nicotine.

This conclusion is based on the assumptions that added formaldehyde uniformly dilutes the metabolic formaldehyde pool and that there is a substantial amount of HCHO in the incubation mixture throughout the experiment. We have not investigated the issue of formaldehyde pools. The question concerning the presence of excess formaldehyde at the end of the incubation was verified by repeating the incubation of 1-NCD_3 with a tenfold excess of added HCHO and cyanide ion. One equivalent of nornicotine-2′,3′,3′-d_3 (**6**-2′,3′,3′-d_3) was then added at the end of the incubation as a trap for formaldehyde. The ether extract obtained from this experiment was analyzed by GC–EIMS using the selected ion recording (SIR) technique.[10] The ions monitored (*m/e* 109, 111, 112, and 114; ions iv, iv-NCD_2–, iv-2′,3′,3′-d_3, and iv-d_5) correspond to the base peaks of each of the isotopically labeled variants of *N*-(cyanomethyl)nornicotine expected from the incubation. The base peaks (M^+ – pyridine) were selected for monitoring, since each isotopically labeled variant of **12** gives rise to ion current predominantly at a single mass in the region of the base peak, while the presence of M^+ – H and M^+ – D, as well as excess deuterium incorporation in the pyridine ring of the deuterated nicotine analogues,[12] causes mutual interference in the molecular ion region. The results of the SIR analysis are summarized in column A of Table II. The large ion current at *m/e* 112 (ion iv-2′,3′,3′-d_3) establishes that a large amount of *N*-(cyanomethyl)nornicotine-2′,3′,3′-d_3 (**13**-2′,3′,3′-d_3) was formed; a GC tracing showed that all of the added nornicotine-2′,3′,3′-d_3 was consumed. This indicates that at least 1 equiv of formaldehyde remained at the end of the incubation. Furthermore, the large *m/e* 112 (iv-2′,3′,3′-d_3) to 114 ion (iv-d_5) ratio (20:1) shows that a large excess of the unlabeled formaldehyde was still present. The ion currents recorded at *m/e* 109 and 111 (ions iv and iv-NCD_2–) were of equal intensity, indicating that cyanomethyl- and cyanomethyl-d_2-nornicotine (**12** and **12**-NCD_2–, respectively) were formed in approximately equal amounts. This agrees with the earlier findings that **12** is formed in part by the direct pathway.

We carried out a variation on the above experiment to further substantiate the validity of this conclusion. Instead of adding excess formaldehyde as a trap for metabolic nornicotine, labeled nornicotine was added as a trap for metabolically formed formaldehyde. An equimolar mixture of 1-NCD_3 and nornicotine-2′,3′,3′-d_3 were coincubated, and the amount of *N*-(cyanomethyl-d_2)nornicotine (**12**-NCD_2–) formed by the direct pathway was compared to the amount of the d_5 analogue (**12**-d_5) formed by the indirect pathway (Scheme II). SIR analysis of ions at *m/e* 111 and 114 (ions iv-NCD_2– and iv-d_5) measured the relative amounts of **12**-NCD_2– and **12**-d_5 formed. Column B in Table II summarizes the results. Approximately twice the amount of the cyanomethyl compound was formed by the direct pathway (*m/e* 111, 100%) compared to the indirect pathway (*m/e* 114, 53%), which we interpret as evidence for the intermediary role of the methyleniminium ion **13** in the in vitro metabolism of nicotine by rabbit liver preparations. A SIR analysis for nornicotine at *m/e* 70 and 73 (the base peak, M^+ – pyridine) established that the mixture of nornicotine-2′,3′,3′-d_3 and nornicotine left at the end of the experiment was approximately 6:1, respectively.

Close examination of the EIMS of the various deuterated 5′-cyanonicotines and deuterated cyanomethylnornicotines obtained from the incubations revealed only the expected numbers of deuterium atoms in all cases. This evidence rules out the possibility of equilibria between the three possible iminium ions [$\Delta^{1(2)}$-iminium ion, $\Delta^{1,(5)}$-iminium ion, and *N*-methyleniminium ion], since such equilibria would result in an exchange of the α-C deuterons with the protons from the medium. Incubations in the presence of cyanide ion using 10000*g* preparations were carried out in isotonic KCl (measured pH of incubate = 9) and in phosphate buffer (measured pH of incubate = 7.4). Although there were large variations in the relative amounts of 5′-cyanonicotine and *N*-(cyanomethyl)nornicotine formed from animal to animal, no systematic changes were observed between those incubations carried out at pH 9 and those carried out at pH 7.4. Neither were there any systematic changes observed in going from incubations carried out with racemic nicotine-2′,5′,5′-d_3 and racemic nicotine-*N*-*methyl*-d_3 to incubations carried out with samples having the natural configuration (*S*)-nicotine and (*S*)-nicotine-5′,5′-d_2.

Experimental Section

The syntheses of the tobacco alkaloids and their deuterated analogues used in these studies have been previously published.[11] Glassware was cleaned in a nitric acid bath before used in metabolic studies. Male Dutch black rabbits 6 months to 1 year old were used. The animals were stunned by a blow to the neck, followed by decapitation. Their livers were quickly removed and rinsed in ice-cold 1.15% KCl. Liver tissue (12.5 g) was minced in 25 mL of cold isotonic KCl solution or pH 7.4 phosphate buffer solution and homogenized using a Potter-Elvehjem Teflon pestle homogenizer. The homogenates were centrifuged at 10000*g* for 30 min in a Sorvall RCZ-B refrigerated centrifuge at 0–4 °C. The supernatant fractions were recentrifuged at 100000*g* for 1 h in a refrigerated Spinco Model L centrifuge at 0–4 °C. The sediment (the microsomal pellet) was rinsed three times with the phosphate buffer and was resuspended in the amount of ice-cold pH 7.4 phosphate buffer needed to make 1 mL of solution correspond to 0.5 g of liver.

Each incubate (10 mL total volume) contained 8 mL of either 10000*g* supernatant or microsomal fraction (0.5 g of liver/mL), magnesium chloride (1.43 mg, 1.5 mM), sodium cyanide (5 mg, 10 mM), NADPH (added at 20-min intervals in 8-mg portions), and nicotine (810 μg, 0.5 mM). Incubations were carried out at 37 °C in air for 1 h using a metabolic shaker. When the 10000*g* supernatant was used as the enzyme source, the incubation mixture was preincubated at 0 °C for 15 min before the substrate was added and incubated at 37 °C.

The incubation was stopped by chilling in an ice bath, followed by a direct extraction with fresh diethyl ether at 0 °C. An

acid–base (0.1 M HCl, concentrated K_2CO_3) extraction was then done to remove nonbasic substances. The base extracts were dried over anhydrous sodium sulfate, and the volume was reduced to 0.1 mL under a stream of nitrogen. This solution was analyzed by GC–MS on 2% Dexyl (160 °C for 1 min, then increasing 3.5 °C/min).

Electron-impact mass spectra (EIMS) were obtained by gas chromatography–mass spectrometry (GC–MS) on an AEI MS-12 mass spectrometer which is interfaced to a PDP 8/I computer using the DS-30 software. An Infotronics 2400 gas chromatograph using helium as the carrier gas is interfaced to the mass spectrometer via a Biemann–Watson molecular separator. Mass spectra were taken with an 8-kV accelerating voltage, a trap current of 500 μA, an electron beam energy of 70 eV, a source temperature of 200 °C, and a resolving power of 1200.

SIR analyses were obtained with the above GC–MS system modified for analog SIR operation with 4-channel output to a Rikadenki KA-42 four-pen recorder.[10]

Control Experiments. Several control incubations were done for the nicotine studies using exactly the reagents and procedures described above, except that the liver preparation was heated for 10 min on a steam cone before the cofactors and substrate were added.

References and Notes

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Antiallergy Agents. 1. 1,6-Dihydro-6-oxo-2-phenylpyrimidine-5-carboxylic Acids and Esters

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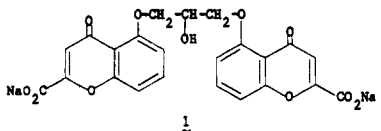
The synthesis of some 1,6-dihydro-6-oxo-2-phenylpyrimidine-5-carboxylic acids and esters with potent oral and intravenous antiallergic activity against passive cutaneous anaphylaxis in the rat is described. Requirements for high activity include a free NH group in the pyrimidinone nucleus and a small to medium size ortho alkoxy or alkenyloxy group on the phenyl ring. It is suggested that in the case of the highly active compounds hydrogen bonding occurs between a nitrogen of the pyrimidine ring and the ethereal oxygen. The nature of this bonding and its possible contribution to an optimum configuration for the molecules is discussed.

The introduction in 1968 of the mediator release inhibitor disodium cromoglycate (DSCG, 1) provided an important new and safe method for the prophylactic and adjunctive treatment of allergic disease such as bronchial asthma. DSCG (1), however, is not absorbed orally to any

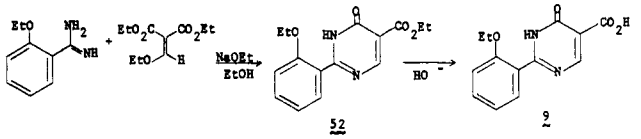


1

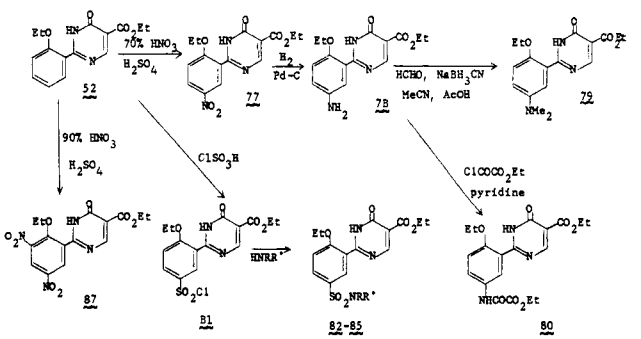
significant extent, and for the treatment of asthma must be inhaled as a powder.[1] Extensive efforts have already been made to find an orally effective alternative.[2]

In this paper we describe the synthesis and structure–activity relationships of some 1,6-dihydro-6-oxo-2-phenylpyrimidine-5-carboxylic acids and esters (Tables I and II), some of which show potent antiallergic activity by both oral and intravenous routes of administration.[3] Just as we were completing our work some related, although considerably less potent, pyrimidine-5-carboxylic acid antiallergy agents were disclosed.[4] 2-Phenylpyrimidine-5-carboxylic acids and esters with antiinflammatory, antipyretic, and analgetic activity have also been reported.[5]

Scheme I



Scheme II



Chemistry. Most of the monosubstituted and some of the disubstituted acids and esters in Tables I and II were

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