Molar Volume Relationships and the Specific Inhibition of a Synaptosomal Enzyme by Psychoactive Cannabinoids

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The ability of a number of lipophilic compounds to inhibit the mouse-brain synaptosomal enzyme acyl coenzyme A:lysophosphatidylcholine acyltransferase has been measured in vitro. Psychoactive cannabinoids inhibit the enzyme at concentrations much lower than is predicted from their capacity to act as lipid-soluble anesthetics. Nonpsychoactive cannabinoids do not show specific inhibition. Molar volume relationships are used to show that, while all lipid-soluble molecules exert some inhibitory effect in proportion to their ability to dissolve in biological membranes, psychoactive cannabinoids have an inhibitory effect greatly in excess of their anesthetic potency. The isoprenoid convulsant thujone has been suggested to have psychoactivity similar to cannabinoids but does not mimic the cannabinoids in inhibiting the synaptosomal enzyme. Molar volumes and specific interactions are used in structure-activity correlations which yield information on the relative concentrations of biophase in drug-responsive systems and the specificity of membrane-active drugs.

The psychoactive constituents of marihuana are the cannabinoids, the principal component being (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC). A variety of other natural and synthetic cannabinoids of varying psychoactive potencies which depend on side-chain substitutions and ring modifications have been described. One explanation of cannabinoid psychoactivity that has gained wide acceptance is the "partial anesthetic" hypothesis[2] and is based on the ability of cannabinoids to cause membrane perturbations similar to those seen for many lipid-soluble anesthetics.[3] We report here the inhibition of a membrane-bound enzyme in synaptosomes by psychoactive cannabinoids at concentrations well below those required to produce nonspecific anesthetic effects or molar volume dependent membrane perturbations.

The high lipid solubility of the cannabinoids would suggest that their action be at least partly mediated at the level of cell membranes. A variety of nonspecific effects on cannabinoids on cell membrane processes have been described, including inhibition of lymphocytic transformation,[4] lysosomal lysis,[5,6] mitochondrial disruption,[7,8] and the uptake of putative neurotransmitters by mouse-brain synaptosomes.[9,10] Recently some membrane processes have been reported to respond specifically to psychoactive cannabinoids, in particular, the uptake of serotonin by mouse-brain synaptosomes[10] and the inhibition of T-lymphocytic acyltransferase.[11] The latter studies are extended in the present paper to mouse-brain synaptosomal acyltransferase activity, which we have shown to be inhibited by Δ^9-THC given to mice in single doses in vivo.[12] The plasma membrane-bound enzyme acyl-CoA:lysophosphatidylcholine acyltransferase (LPC-acyltransferase, E.C. 2.3.1.23) is thought to be responsible for regulating the proportion of saturated fatty acids present in phosphatidylcholines (PC) in the plasma membrane and may play an important role in the maintenance of membrane structure and integrity.[13] It has been demonstrated that this enzyme in mouse lymphocytes can be inhibited by Δ^9-THC at low concentrations (K_i = 0.35 μM).[14] While other lipids including psychoinactive cannabinoids are capable of perturbing membrane bilayers and at high concentrations can inhibit the lymphocytic LPC-acyltransferase, only Δ^9-THC can inhibit enzyme at micromolar concentrations.[11] In the present paper we demonstrate that a similar enzyme activity in mouse-brain synaptosomes is also inhibited by low levels of psychoactive cannabinoids and that, while many lipid-soluble substances can inhibit the enzymes at high concentrations, in proportion to their nonspecific ability to cause anesthesia, cannabinoids can inhibit the synaptosomal enzymes at much lower concentrations in the order of their psychoactivity.

Molar volume correlations are used in this study to distinguish the nonspecific inhibition of the synaptosomal enzymes shown by all lipid substances, in proportion to their ability to perturb lipid membranes, from the specific inhibition seen for psychoactive cannabinoids. The ability of lipid-soluble molecules to induce anesthesia is known to be closely related to their molar volumes[15] and to their ability to protect erythrocytes against hypotonic lysis (AH_{50}).[16] Using molar volume correlations, the ability of cannabinoids and other compounds to inhibit synaptosomal acyltransferase is compared with their ability to expand erythrocytic membranes.

Experimental Section

Synaptosomes were prepared from mouse brain as described by Cotman[17] and stored at 10–15 times the final concentration at −10 °C in 0.95 M phosphate buffer, pH 7.4. Samples were thawed and diluted with Krebs-Ringer phosphate buffer to 0.5 mg of protein before use in the LPC-acyltransferase assays. Protein was determined according to Lowry et al.[18]

LPC-acyltransferase activity was determined as described previously,[11] using as substrates 200 nmol of [^{32}P]lysophosphatidylcholine and 100 μmol of oleoyl-CoA per milliliter of incubation mixture. Synaptosomes were preincubated for 30 min at 37 °C with 0.05 mL of dimethyl sulfoxide containing the test lipid. Controls were preincubated with dimethyl sulfoxide only. Acyltransferase activity at each concentration of lipid was compared with control values, and the concentration which produced half-maximal inhibition was defined as the K_i for that compound.

Lipids were purchased from Sigma Chemical Co., St. Louis, MO, except for cannabinoids which were generously provided by National Health and Welfare Canada. Thujone was obtained from Aldrich Chemical Co., Milwaukee, WI, and long-chain alcohols were purchased from Applied Science Inc., State College, PA.

The concentration of a compound which provides 50% stabilization of erythrocytes against hypotonic lysis is defined as the AH_{50} for that compound. These values were measured as described previously for cannabinoids, retinol, and thujone[11] or obtained from the data of Roth and Seeman.[16] The *characteristic volumes* (m^3 mol^{-1}) are estimates of the actual molar volumes (i.e., the molar volumes at absolute zero) and are obtained using the method of McGowan[19] by division of the calculated parachors[20] in cgs units by 2.835×10^6. Details of the characteristic volume (V_x) relationships are given in the Results and Discussion.

Results and Discussion

In this study two types of membrane activity have been measured for a number of highly hydrophobic molecules over a wide range of molecular size. The first biological activity is the inhibition of a mouse-brain synaptosomal

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enzyme, LPC-acyltransferase, by a number of membrane-soluble lipids including psychoactive and nonpsychoactive cannabinoids. The second type of activity is related to the anesthetic potency of the lipid and is its ability to stabilize erythrocytes against hypotonic lysis. The major question asked in this study is whether both of these activities are determined by the hydrophobicity of the molecules as predicted by the partial anesthetic hypothesis of cannabinoid action. Alternatively, is there evidence of specific interactions for psychoactive cannabinoids?

To answer these questions we have correlated both activities with the molar volumes of the compounds, a treatment which is successful in the prediction of physical toxicity of which the best known general examples are anesthesia and narcosis.[21] The following discussion establishes the basis for the molar volume parameters used in this study. While the approach has been used previously to predict physical toxicity, this is the first application to the diagnosis of specific drug interactions.

Equation 1 has been used[22] to predict partition coefficients (x), where x = (concn of compd in water)/(concn of compd in organic phase). Liquid water has an intermolecular structure and kV_x is a measure of the extent to which this structure is broken up by the compound dissolved in it. The characteristic volume, V_x, is an estimate of the molar volume when the molecules are not in motion (i.e., at absolute zero temperature) and can be obtained by the addition of factors.[19,22,23] V_x (SI units, m^3 mol^{-1}) equals the parachor (in cgs units as used in most earlier studies[20]) divided by 2.835×10^6. As a first approximation, k is the same for all nonaqueous phases and equals 36 000 mol m^{-3}. If the compound is associated or forms a complex with some component of either phase, this is taken into account by the term E_B which is an interaction term and is constant for a given chemical grouping.[24] Equation 1 has proved useful for the correlation and prediction of physical toxicity in which biological activity depends on the active compound reaching a certain definite concentration (C_B) in some nonaqueous biophase.[21,24,25] If C_t is the toxic concentration required in the aqueous phase to produce a certain manifestation of toxicity, given at C_B in the biophase, then log x in eq 1 can be replaced by log (C_t/C_B). A plot of -log C_t against $36\,000V_x - E_B$ should give a line of slope 45°, and the intercept of this line when $36\,000V_x + E_B$ is zero equals -log C_B. In physical toxicity it has been found[24] that E_B for compounds with a carbonyl, ester, aliphatic hydroxyl, or aliphatic ether is about 1.2 and for compounds with a phenolic hydroxyl or aromatic ether about 0.6.

The concentration, C_t, used for eq 1 must be that for the aqueous phase only. However, toxicities are usually measured in systems consisting of aqueous plus nonaqueous phases, and this is especially true for studies on membrane-active drugs. Equation 1 will be inadequate for a system in which the concentration measured includes a nonaqueous phase containing any appreciable proportion of the compound. Equation 1 can be modified so that it can be applied to a system containing an aqueous and a nonaqueous phase. The modification has already been applied to the injection of physically toxic compounds into animals[25] and has been more recently used by Hyde.[26,27] The modified eq 2 can be written

$$C_t' = \frac{C_B x_f}{100 d_f} \times 10^{E_f - E_B} + \frac{C_B x_w}{100 d_w} \times 10^{-(kV_x - E_B)} \qquad (2)$$

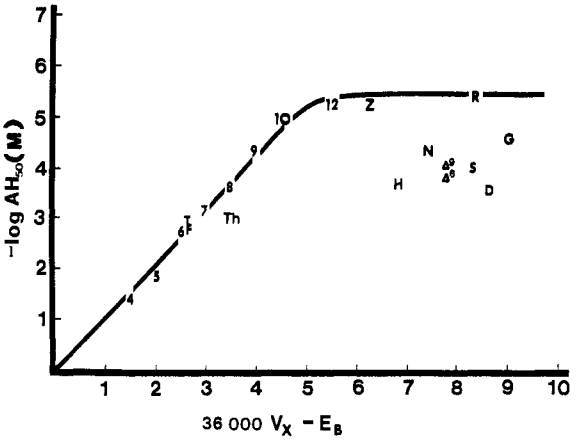

Figure 1. Molar volume and antihemolytic activities. The antihemolysis potency (AH_{50}), the characteristic volume (V_x), and the interaction term (E_B) are defined in the text. The compounds tested are 1-butanol (**4**), 1-pentanol (**5**), 1-hexanol (**6**), 4-chlorophenol (F), 2-chlorophenol (T), 1-heptanol (**7**), thujone (Th), 1-octanol (**8**), 1-nonanol (**9**), 1-decanol (**10**), 1-dodecanol (**12**), chlorpromazine (Z), retinol (R), 11-OH-Δ^9-THC (H), cannabinol (N), Δ^9-THC (Δ^9), Δ^8-THC (Δ^8), synhexyl (S), cannabidiol (D), and cannabigerol (G). The line drawn is that described by eq 4 in the text.

where C_t' is the toxic dose in mol kg^{-1} of body weight of the animal, the animal body consisting of x_w% by weight aqueous phase (density, d_w) and x_f% by weight nonaqueous phase (density, d_f) which includes the biophase. The term E_f was introduced to account for interactions in the nonaqueous layer. The other terms in eq 2 are the same as those in eq 1 and indeed the two equations are identical if $x_f = 0$ and $x_w = 100$. Since $C_t = C_t'$ multiplied by the density of the system, eq 2 can be written as eq 3

$$C_t = A + B \times 10^{-36\,000 V_x} + E_B \qquad (3)$$

if it is assumed that $E_f - E_B$ is the same for all toxic compounds. This assumption is in accord with previous findings.[22-24] A and B are the constants for the system, and the ratio of A/B equals (volume of nonaqueous phase in the system)/(volume of aqueous phase in the system). Equation 3 allows the comparison of biphasic systems containing different amounts of nonaqueous phase, such as comparisons in the present study between erythrocytic and synaptosomal suspensions. The proportion of nonaqueous phase in a system can be expected to have a marked effect on the toxic concentration (C_t) of a given drug in that system.

Equation 3 has been used in the present study to correlate the capacity of hydrophobic molecules to expand erythrocyte membranes (AH_{50} values) and to compare this effect with membrane enzyme inhibition (K_i values). Figure 1 shows that smaller molecular weight lipids give increasing protection of the erythrocytic membrane against hypotonic lysis, as predicted for a physical toxicity effect and in accordance with the molar volume hypothesis of anesthetic action. For these compounds whose anesthetic potency increases with molar volume the points fall close to a line of slope 45°. However, for compounds of larger molar volume than dodecanol, i.e., those compounds which are so insoluble in water that they are considered to be almost entirely dissolved in the small amount of nonaqueous phase, there is a limiting value for the AH_{50}.

$$AH_{50} = (5.3 \times 10^{-6}) + (0.60 \times 10^{-36\,000 V_x + E_B}) \qquad (4)$$

The agreement is good since E_B can at present be only

Table I. Molar Volumes and Biological Activities

compd[a]	V_x[b]	E_B	$36\,000V_x - E_B$	$-\log AH_{50}$[c]	$-\log K_i$
octanol (8)	1.295×10^{-4}	1.2	3.46	3.63	2.77
nonanol (9)	1.435×10^{-4}	1.2	3.97	4.39	3.24
decanol (10)	1.576×10^{-4}	1.2	4.55	5.00	3.77
dodecanol (12)	1.858×10^{-4}	1.2	5.48	5.27	3.39
4-chlorophenol (F)	8.975×10^{-5}	0.6	2.63	3.13	2.74
2-chlorophenol (T)	8.975×10^{-5}	0.6	2.63	2.94	2.48
retinol (R)	2.865×10^{-4}	1.2	8.38	5.49	3.82
thujone (Th)	1.132×10^{-4}	1.2	3.54	3.00	3.15
chlorpromazine (Z)	2.406×10^{-4}	2.4	6.26	5.09	3.10
Δ^9-THC (Δ^9)	2.687×10^{-4}	1.8	7.87	4.10	6.52
Δ^8-THC (Δ^8)	2.687×10^{-4}	1.8	7.87	3.85	4.24
cannabinol (N)	2.601×10^{-4}	1.8	7.56	4.40	3.26
cannabigerol (G)	2.861×10^{-4}	1.2	9.10	4.66	3.59
11-OH-Δ^9-THC (H)	2.746×10^{-4}	3.0	6.89	3.72	4.07
synhexyl (S)	2.828×10^{-4}	1.8	8.38	4.03	3.60

[a] Symbols in parentheses represent corresponding points from Figures 1 and 2. [b] Calculated as described in ref 19. [c] Values for octanol, nonanol, decanol, 4-chlorophenol, and 2-chlorophenol are from the data of Roth and Seeman.[15] Other values were determined as described previously.[11]

given very approximate values (Table I). E_f has been ignored for the present but this does not affect our conclusions. E_f has been found to be −0.4 for the alcoholic hydroxyl group in the total animal body fat.[25] Values of E_B are additive for compounds containing more than one of these groups unless the groups are close together. All groups for which E_B values can be allotted are included.[24]

Figure 1 indicates that the cannabinoids and other large hydrophobic molecules known to have large partition coefficients for the system biophase-water do not cause a membrane expansion consistent with their molar volume. It has long been recognized that such large molecules are above the anesthetic cutoff in correlations of anesthetic potency and molar size. Figure 1 demonstrates that they are also too large to produce erythrocyte-membrane expansion in proportion to their size, despite their ability to partition into membranes readily.[28] In addition, eq 2 and 3 enable us to estimate the proportion of nonaqueous phase to aqueous phase in the experimental system, since the ratio of A/B is equal to $(x_f/d_f)(d_w/x_w)$, which equals (vol of nonaqueous phase)/(vol of aqueous phase). In the example just given this equals $(5.3 \times 10^{-6})/0.60 = 8.8 \times 10^{-6}$, so that in a liter of the erythrocytic suspension there would be less than 100 μL of the nonaqueous phase. This small amount of nonaqueous phase can account for the bending of the line in Figure 1. If the nonaqueous phase was to approach zero concentration then the line would be straight with a slope of 45°, but in Figure 1 it bends toward the horizontal, the horizontal region corresponding to a constant concentration of $A = 5 \times 10^{-6}$ mol/L in the system, i.e., in 8.8×10^{-6} L of nonaqueous phase. Thus C_B is $(5.3 \times 10^{-6})/(8.8 \times 10^{-6})$ mol/L (i.e., B) and $\log C_B = -0.2$. The following values of $\log C_B$ have been given previously:[21] for narcosis of tadpoles, −0.2, and for narcosis of mammals, −0.3. It is of interest that all these values for anesthetic effects are so close. From eq 4 the volume of the biophase in the erythrocytic suspension was estimated to be less than 100 μL/L. This is close to an estimate of 60 μL/L which is obtained from the measured hematocrit of the blood, its dilution, and its lipid content of about 330 mg %.

We have previously shown that psychoactive cannabinoids have the ability to inhibit mouse lymphocytic membrane LPC-acyltransferase at concentrations much lower than for nonpsychoactive cannabinoids and other lipids acting at anesthetic concentrations.[11] In the previous study we correlated AH_{50} potencies with K_i values for all lipids except psychoactive cannabinoids. In Figure 2 it can be seen that the inhibition of mouse-brain synaptosomal

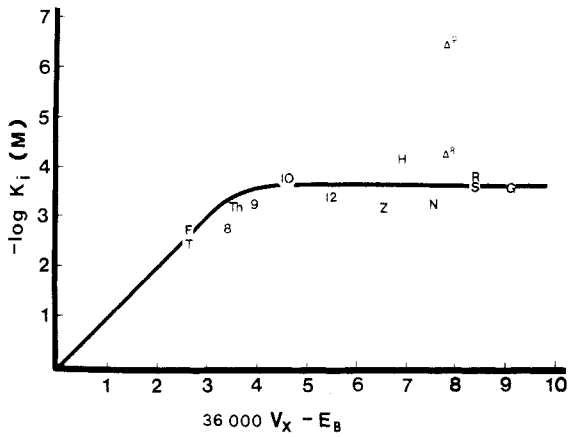

Figure 2. Molar volume and acyltransferase inhibition. The concentration for 50% of maximal inhibition of LPC-acyltransferase (K_i) was measured as described in the Experimental Section, and the V_x and E_B terms are defined in the text. The compounds tested are 4-chlorophenol (**F**), 2-chlorophenol (T), 1-octanol (**8**), thujone (Th), 1-nonanol (**9**), 1-decanol (**10**), 1-dodecanol (**12**), chlorpromazine (**Z**), 11-OH-Δ^9-THC (H), cannabinol (N), Δ^9-THC (Δ^9), Δ^8-THC (Δ^8), synhexyl (S), retinol (R), and cannabigerol (G). The line drawn is that described by eq 4 in the text.

LPC-acyltransferase activity in vitro shows a very similar specificity for psychoactive cannabinoids, which show K_i values (concentrations for 50% inhibition) much lower than for nonpsychoactive cannabinoids and other lipids. As for the inhibition of the lymphocytic enzyme, the potency of Δ^9-THC is about 100 times greater than that of the less psychoactive Δ^8-THC and 11-OH-Δ^9-THC, and these are about 10 times more potent than the nonpsychoactive compounds in inhibition of the brain synaptosomal enzyme. Clearly there is little correlation between the membrane-expanding capacity of the psychoactive cannabinoids as measured by the AH_{50} and their ability to inhibit the synaptosomal enzyme.

The isoprenoid thujone, a compound found in the liqueur absinthe and which is thought to be responsible for the toxic, convulsant properties of absinthe, has certain stereochemical similarities to Δ^9-THC. On the basis of these similarities it has been proposed that thujone may have cannabinoid-like psychoactivity.[29] For this reason we included thujone in the determination and correlation of K_i values for LPC-acyltransferase in mouse-brain synaptosomes. As seen in Figures 1 and 2, thujone does not have the capacity to expand erythrocytic membranes nor

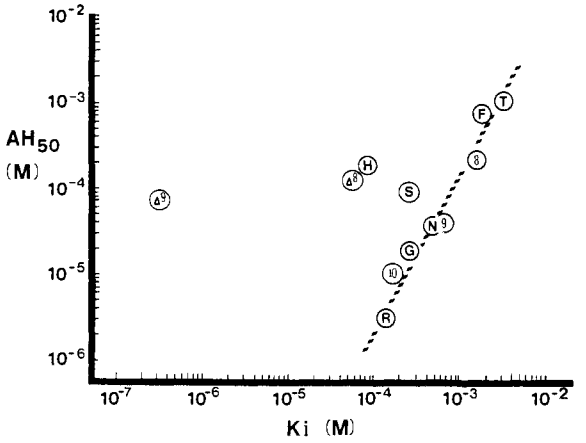

Figure 3. The inhibition of LPC-acyltransferase of mouse synaptosomes in vitro as a function of the antihemolytic potency of inhibitors. The terms K_i and AH_{50} are defined in the text. The compounds shown are Δ^9-THC (Δ^9), Δ^8-THC (Δ^8), 11-OH-Δ^9-THC (H), synhexyl (S), retinol (R), 1-decanol (**10**), cannabigerol (G), cannabinol (N), 1-nonanol (**9**), 1-octanol (**8**), 4-chlorophenol (F), and 2-chlorophenol (T). The line shown is a least-squares fit to the noncannabinoid anesthetic compounds and, for this line, the correlation coefficient, r, is 0.994.

the ability to inhibit the membrane enzyme that would be expected of a psychoactive cannabinoid-like compound. From our correlations we conclude that it is unlikely that thujone and the cannabinoids act by similar mechanisms in eliciting neural responses.

The curve shown in Figure 2 corresponds to a form of eq 3 in which $K_i = (2 \times 10^{-4}) + 10^{-36\,000 V_x + E_B}$. From this, A/B = (volume of nonaqueous phase)/(volume of aqueous phase) = 2×10^{-4}, still a small value for the ratio of the biophase but about 30 times larger than the ratio for the AH_{50} determination, corresponding to a much higher concentration of cellular material used in the enzyme assay. A very similar curve is obtained for the plot of K_i values from lymphocytic LPC-acyltransferase inhibition studies reported previously,[11] suggesting that the biophase concentration is similar for both systems and that the mechanism of inhibition is analogous.

According to Hollister[30] Δ^9-THC can be assigned a relative psychoactivity of 100, Δ^8-THC 75, synhexyl 30, and cannabinol and cannabigerol 0. The cannabinoids appear to deviate from the membrane perturbation line in direct proportion to their relative psychoactivities (Figure 3). The most potent cannabinoid tested, Δ^9-THC, shows the greatest deviation from the line, the moderately psychoactive Δ^8-THC and 11-OH-Δ^9-THC deviate less from the line, the slightly active Δ^{6a}-THC falls close to the line, and the psychoinactive cannabinol and cannabigerol show no deviation. Estimates of psychoactive potencies are generally somewhat subjective and depend on a variety of variables such as species, route of administration, and extent of metabolism to other compounds of variable psychoactivity. While early reports of the potency of 11-OH-Δ^9-THC suggested that it was more psychoactive than Δ^9-THC, more recent studies indicate that it is less psychoactive but is taken up more readily by the brain in vivo.[31]

The major conclusion to be drawn from these correlations is that inhibition of synaptosomal LPC-acyltransferase activity below concentrations of 1.6×10^{-4} M is due to a process which is more specific than the perturbation of the surrounding lipid bilayer. The only compounds tested which demonstrated this specific inhibition were the psychoactive cannabinoids. The difference is not explicable in terms of the partition coefficients, water solubilities, or molar volumes of these compounds.

The cannabinoids have been shown to accumulate in the synaptosomes upon in vivo administration[32] and the high lipid solubility of the cannabinoids suggests the synaptosomal membrane as a possible site of action. In vivo experiments have shown that single doses of Δ^9-THC can significantly inhibit mouse synaptosomal and lymphocytic LPC-acyltransferase activity.[12] The inhibition of a membrane-bound enzyme responsible for maintenance of membrane integrity may have implications in neurotransmitter synthesis and release or in membrane excitation. The correlation between psychoactive potency of the cannabinoids and the degree of specific inhibition of LPC-acyltransferase activity suggests that further investigation of this inhibition may lead to an understanding of the mechanism by which cannabinoids exhibit psychoactivity.[33]

References and Notes

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(2) W. D. M. Paton in "Molecular Mechanisms in General Anaesthesia", M. J. Halsey, R. A. Millar, J. A. Sutton, Eds., Churchill-Livingstone, London, 1974, p 48.
(3) D. K. Lawrence and E. W. Gill, *Mol. Pharmacol.*, 11, 595 (1975).
(4) G. G. Nahas, N. Sucia-Foca, J.-P. Armand, and A. Morishima, *Science*, **183**, 419 (1974).
(5) A. Raz, A. Schurr, A. Livne, and R. Goldman, *Biochem. Pharmacol.*, **22**, 3129 (1973).
(6) R. S. Britton and A. Mellors, *Biochem. Pharmacol.*, **23**, 1342 (1974).
(7) T. A. Bino, A. Chari-Bitron, and A. Shahar, *Biochim. Biophys. Acta*, **288**, 195 (1972).
(8) J. M. Mahoney and R. A. Harris, *Biochem. Pharmacol.*, **21**, 1217 (1972).
(9) S. P. Banerjee, H. Snyder, and R. Mechoulam, *J. Pharmacol. Exp. Ther.*, **194**, 74 (1975).
(10) M. Hershkowitz, R. Goldman, and A. Raz, *Biochem. Pharmacol.*, **26**, 1327 (1977).
(11) J. H. Greenberg and A. Mellors, *Biochem. Pharmacol.*, **27**, 329 (1978).
(12) L. A. Huszar, J. H. Greenberg, and A. Mellors, *Mol. Pharmacol.*, **13**, 1086 (1977).
(13) W. E. M. Lands and P. Hart, *J. Biol. Chem.*, **240**, 1905 (1965).
(14) J. H. Greenberg, M. E. Saunders, and A. Mellors, *Science*, **197**, 475 (1977).
(15) S. Roth and P. Seeman, *Biochim. Biophys. Acta*, **255**, 207 (1972).
(16) H. Machleidt, S. Roth, and P. Seeman, *Biochim. Biophys. Acta*, **255**, 178 (1972).
(17) C. W. Cotman, *Methods Enzymol.*, **29**, 445 (1974).
(18) O. H. Lowry, N. J. Rosebraugh, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
(19) J. C. McGowan, *Recl. Trav. Chim. Pays-Bas*, **75**, 193 (1956).
(20) A. Leo, C. Hansch, and C. Church, *J. Med. Chem.*, **12**, 766 (1969).
(21) J. C. McGowan, *J. Appl. Chem.*, **2**, 651 (1952).
(22) J. C. McGowan, *J. Appl. Chem.*, 4, 41 (1954).
(23) J. C. McGowan, *J. Appl. Chem.*, **2**, 323 (1952).
(24) J. C. McGowan, P. N. Atkinson, and L. H. Ruddle, *J. Appl. Chem.*, **16**, 99 (1966).
(25) J. C. McGowan, *SCI Monogr*, **No. 29**, 41 (1968).
(26) R. M. Hyde, *J. Med. Chem.*, 18, 231 (1975).
(27) R. M. Hyde, *Chem. Ind. (London)*, 859 (1977).
(28) P. Seeman, M. Chau-Wong, and S. Moyyen, *Can. J. Physiol. Pharmacol.*, **50**, 1193 (1972).
(29) J. del Castillo, M. Anderson, and G. M. Rubottom, *Nature (London)*, **253**, 365 (1975).
(30) L. E. Hollister, *Pharmacology*, 11, 3 (1974).
(31) M. Perez-Reyes, J. Simons, D. Brine, G. L. Kimmel, K. H. Davies, and M. E. Wall in "Marihuana: Chemistry, Biochemistry, and Cellular Effects", G. G. Nahas, Ed.,

Springer-Verlag, New York, N.Y., 1976, p 179.
(32) R. W. Coburn, L. K. Y. Ng, L. Lemberger, and L. L. Kopin, *Biochem. Pharmacol.*, **23**, 873 (1974).
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Synthesis and Antiviral and Enzymatic Studies of Certain 3-Deazaguanines and Their Imidazolecarboxamide Precursors

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Due to the varied and potent biological activity of 3-deazaguanine (**20**), 3-deaza-7-β-D-ribofuranosylguanine, 3-deazaguanosine (**22**), and 3-deazaguanylic acid (**24**), several 3-deazaguanines, mainly with modification in the 7 and 9 positions, were prepared. 7-(5-Deoxy-β-D-ribofuranosyl)- and 7-(tetrahydropyran-2-yl)-3-deazaguanine (**12** and **13**) were obtained by ammonolysis of the corresponding 1-substituted methyl 4-(cyanomethyl)imidazole-5-carboxylates, **6** and **8**, and subsequent in situ cyclization. 9-(5-Deoxy-β-D-ribofuranosyl)- and 9-(tetrahydropyran-2-yl)-3-deazaguanine (**14** and **15**) were obtained by ammonolysis of the corresponding 1-substituted methyl 5-(cyanomethyl)imidazole-4-carboxylates, **5** and **7**, to provide 1-(5-deoxy-β-D-ribofuranosyl)- and 1-(tetrahydropyran-2-yl)-5-(cyanomethyl)imidazole-4-carboxamides (**9** and **10**, respectively), which were subsequently cyclized with aqueous potassium carbonate. Methyl 4-(cyanomethyl)-1- or -3-(5-deoxy-2,3-di-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylates, **5** and **6**, were obtained from the stannic chloride catalyzed condensation of methyl 5(4)-(cyanomethyl)-1-(trimethylsilyl)imidazole-4(5)-carboxylate (**2**) and 5-deoxy-1,2,3-tri-*O*-acetyl-β-D-ribofuranose (**3**). Methyl 4(5)-(cyanomethyl)imidazole-5(4)-carboxylate (**1**) and dihydropyran in the presence of acid provided the tetrahydropyran-2-yl derivatives **7** and **8**. The in vitro antiviral and antibacterial activity of these 3-deazaguanines, their imidazolecarboxamide precursors, and several acetylated derivatives were compared with 3-deazaguanine (**20**), 3-deazaguanosine (**22**), and 3-deazaguanylic acid (**24**), their imidazolecarboxamde precursors, 4(5)-(cyanomethyl)imidazole-5(4)-carboxamide (**19**), 5-(cyanomethyl)-1-β-D-ribofuranosylimidazole-4-carboxamide (**21**), and 5-(cyanomethyl)-1-β-D-ribofuranosylimidazole-4-carboxamide 5′-phosphate (**23**), and ribavirin. The most active compounds, **19**, **21**, and **23**, possessed an in vitro antiviral spectrum similar to, but generally less potent than, the corresponding ring-closed compounds **20**, **22**, and **24**. Compound **23** was found to be a potent, specific inhibitor of IMP dehydrogenase. Data are presented which support the antiviral activity of **19**, **21**, and **23** independent of the possible enzymatic cyclization to the corresponding imidazo[4,5-*c*]pyridine.

The recently synthesized[2] 3-deazaguanine [6-aminoimidazo[4,5-*c*]pyridin-4(5*H*)-one, **20**] and its probable metabolites, 3-deazaguanosine [6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one, **22**] and 3-deazaguanylic acid [6-amino-1-β-D-ribofuranosylimidazo-[4,5-*c*]pyridin-4(5*H*)-one 5′-phosphate, **24**], are potent inhibitors of biosynthesis of purine nucleotides[3] and possess marked antiviral[4] and anticancer activity.[3b,5] Furthermore, 3-deaza-7-β-D-ribofuranosylguanine [6-amino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one[2]] has highly significant antibacterial activity[6] against Gram-negative bacteria.

We have continued the study of 3-deazaguanine and its derivatives and now wish to report the synthesis, structure–activity relationships, and biochemical studies of certain 3-deazaguanines and their imidazole precursors which have sugar modifications in the 7 or 9 positions. We have included for comparison the biological activity of several related nucleosides which have been modified in the 6 position, such as the recently prepared[7] 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine-4(5*H*)-thione (3-deaza-6-thioguanosine) and 4,6-diamino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (2-amino-3-deazaadenosine).

Synthesis. The most useful synthetic approach to 3-deazaguanine (**20**) is that reported[2] utilizing the unique ring closure of 4(5)-(cyanomethyl)imidazole-5(4)-carboxamide (**19**) under basic conditions. Thus, we have extended this approach to the synthesis of the 9-substituted 3-deazaguanines **14** and **15** (Scheme I) through their corresponding imidazolecarboxamides **9** and **10**. Unfortunately, liquid ammonia treatment or other milder treatment of 1-substituted methyl 4-(cyanomethyl)-imidazole-5-carboxylates **6** and **8** did not provide the corresponding imidazolecarboxamides which, if formed,[8] cyclized in situ to the 7-substituted 3-deazaguanines **12** and **13**. The difference in reactivity of positional isomers of imidazole **1** can be attributed initially to steric hindrance by the 5-(cyanomethyl) group and the N_3 substituent of imidazoles **6** and **8** toward ammonolysis of the ester group in the 4 position as compared to the ammonolysis of the relatively unhindered ester group in the 5 position of imidazoles[8] **5** and **7**. A similar difference in the reactivity of the 1- and 3-β-D-ribofuranosides of 4(5)-cyano-5(4)-(cyanomethyl)imidazole toward cyclization has previously been noted.[7]

The imidazole carboxylates **5**–**8** were prepared by glycosylation of **1** or its silylated derivative **2**. The initial reaction, **2** → **5** and **6**, involves an anhydrous stannic chloride catalyzed condensation of 5-deoxy-1,2,3-tri-*O*-acetyl-β-D-ribofuranose (**3**) and methyl 1-(trimethylsilyl)-5(4)-(cyanomethyl)imidazole-4(5)-carboxylate (**2**) to provide the positional isomers **5** and **6**. Imidazole **1** and 2,3-dihydropyran (**4**) were allowed to react in the presence of acid to afford tetrahydropyran-2-yl derivatives **7** and **8**.

Several other prodrug-type modifications were obtained by acetylation of **20**–**22**, according to standard procedures, to provide 6-acetamidoimidazo[4,5-*c*]pyridin-4(5*H*)-one (**17**), 5-(cyanomethyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribo-

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