Heterocyclic Tetrazoles, a New Class of Lipolysis Inhibitors[1]

GERALD F. HOLLAND AND JOSEPH N. PEREIRA

Medical Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Connecticut 06340

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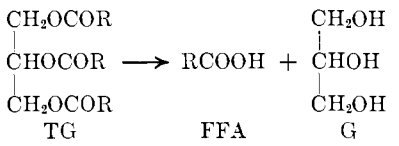
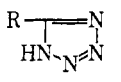
A series of pyridyl-substituted 5-(3-pyridyl)tetrazoles and other 5-(heterocyclic)tetrazoles were prepared and evaluated for lipolysis inhibitory activity. The reaction between heterocyclic nitriles and sodium azide in dimethylformamide provided a convenient synthetic procedure for most of these compounds. They were screened for their ability to inhibit the norepinephrine-induced release of free fatty acids (FFA) from isolated rat adipose tissue and for their ability to depress the fasting plasma FFA levels in the dog. The most active lipolysis inhibitor was 5-(3-pyridyl)tetrazole. Although 5-(3-pyridyl)tetrazole was a much weaker *in vitro* lipolysis inhibitor than nicotinic acid, it depressed plasma FFA levels in the dog for a longer period of time. The relationship be ween inhibiting lipid mobilization from adipose tissue and decreasing plasma lipid levels was developed.

During the past 5 years a wide variety of compounds of varying structures have been examined in these laboratories for their ability to inhibit lipid mobilization (lipolysis inhibition) from adipose tissue.[2] Lipid mobilization involves the net release of free fatty acids (FFA) from the triglyceride-rich adipose tissue stores. It has been firmly established that the FFA are a primary source, *via* hepatic synthesis, of plasma lipoprotein triglycerides.[3,4] Thus, inhibiting triglyceride hydrolysis in adipose tissue would reduce the supply of plasma FFA to the liver, thereby reducing hepatic triglyceride synthesis. The reduced availability of hepatic triglycerides would limit the completion of the major cholesterol transporting unit, the lipoprotein complex. The decrease in the concentration of this transport unit, in turn, restricts the removal of cholesterol from the liver. A normally operative feedback mechanism would then depress hepatic cholesterol synthesis.[5,6]



The pyridine and related heterocyclic acids represent one structural type which we have extensively investigated. Nicotinic acid has been shown to depress the level of plasma FFA after acute administration to man.[7] In addition, it inhibits the norepinephrine-induced release of FFA from isolated adipose tissue.[8,9] It has been suggested that the hypocholesteremic effect of nicotinic acid follows from its lipolysis inhibitory activity.[7] The known rapid metabolism of nicotinic acid[10] could account for the observed short duration of plasma FFA depression which, in turn, could account for the large doses of nicotinic acid required for plasma lipid reduction.[11,12] A lipolysis inhibitor having the same intrinsic activity in isolated adipose tissue as nicotinic acid, but of greater metabolic stability, would be expected to depress plasma FFA for a longer period of time. Such a prolonged depression of plasma FFA would be expected to reduce total plasma lipids more effectively than does nicotinic acid.

The similarity between the acidic character of the carboxyl group and the tetrazole function of 5-substituted tetrazoles (**1**) is well known.[13] For example,



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the ionization constant of 5-phenyltetrazole (pK_a = 4.5) is slightly greater than that of the corresponding carboxylic acid, benzoic acid (pK_a = 5.1).[14] Also of importance, the tetrazole function appears to be metabolically stable.[15] A series of pyridyl- and other heterocyclic tetrazoles were prepared in the hope of finding a lipolysis inhibitor having a longer duration of FFA-depressing activity than that of nicotinic acid.

Synthesis.—The general reaction between pyridyl and other heterocyclic nitriles (**2**) and sodium azide (**3**) served as a convenient procedure for the synthesis of the pyridyl and heterocyclic 5-tetrazoles (**1**). Originally a combination of acetic acid and 1-butanol was used as the solvent for this reaction.[16,17] However, an improved procedure using dimethylformamide, in place of both acetic acid and 1-butanol, gave, on the whole,

higher yields in shorter reaction times.[18] In one case, in the preparation of 5-(3-pyridylmethyl)tetrazole, the reaction between 3-pyridylacetonitrile and sodium azide was only successfully carried out in the acetic acid and 1-butanol solvent combination.

(1) G. F. Holland, F. A. Hochstein, E. R. Pinson, Jr., and J. N. Pereira, presented in part at the session on Recent Developments in Medicinal Chemistry, 10th National Medicinal Chemistry Symposium, Bloomington, Ind., June 28, 1966.

(2) A comprehensive review of adipose tissue can be found in "Handbook of Physiology, Section 5: Adipose Tissue," A. E. Renold and G. F. Cahill, Jr., Ed., American Physiological Society, Washington, D. C., 1965.

(3) R. J. Havel, *Metab. Clin. Exptl.*, **10**, 1031 (1961).

(4) R. J. Havel and A. Goldfien, *J. Lipid Res.*, **2**, 389 (1961).

(5) C. B. Taylor and R. G. Gould, *Circulation*, **2**, 467 (1950).

(6) E. P. Madhava Bhattathiry and M. D. Siperstein, *J. Clin. Invest.*, **42**, 1613 (1963).

(7) L. A. Carlson and L. Orö, *Acta Med. Scand.*, **172**, 641 (1962).

(8) L. A. Carlson, *ibid.*, **173**, 719 (1963).

(9) R. P. Eaton, *Proc. Soc. Exptl. Biol. Med.*, **114**, 599 (1963).

(10) E. Ginoulhiac, L. T. Tenconi, and F. M. Chiancone, *Nature*, **193**, 948 (1962).

(11) R. Altschul, A. Hoffer, and J. D. Stephen, *Arch. Biochem. Biophys.* **54**, 558 (1955).

(12) W. B. Parsons, Jr., R. W. P. Achor, K. G. Berge, B. F. McKensie, and N. W. Barker, *Proc. Staff Meetings Mayo Clinic*, **31**, 377 (1956).

(13) R. M. Herbst in "Essays in Biochemistry," S. Graff, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, pp 141–155.

(14) R. M. Herbst and K. R. Wilson, *J. Org. Chem.*, **22**, 1142 (1957).

(15) D. W. Esplin and D. M. Woodbury, *J. Pharmacol. Exptl. Therap.*, **118**, 129 (1956).

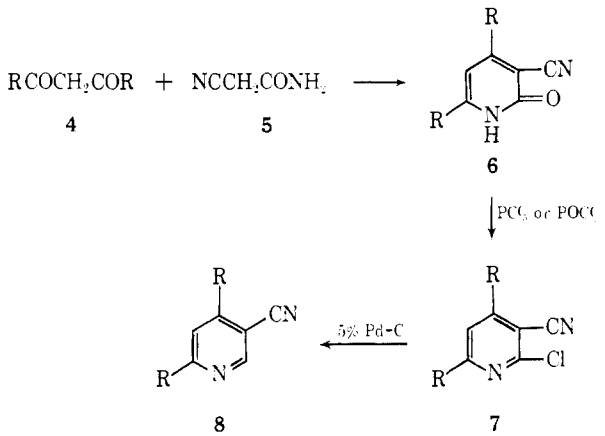
(16) B. Brouwer-van Straaten, D. Solinger, C. van de Westeringh, and H. Veldstra, *Rec. Trav. Chim.*, **77**, 1129 (1958).

(17) J. M. McManus and R. M. Herbst, *J. Org. Chem.*, **24**, 1462 (1959).

(18) W. G. Finnegan, R. A. Henry, and R. Lofquist, *J. Am. Chem. Soc.*, **80**, 3908 (1958).

The majority of the pyridyl and heterocyclic nitriles were prepared by known procedures. The reaction between β-dicarbonyl compounds (**4**) and cyanoacetamide (**5**) to form the corresponding 3-cyano-2(1H)-pyridones (**6**), followed by treatment with PCl_5 or $POCl_3$, and, lastly, dehalogenation with 5% Pd-C was found to be a useful route to 3-cyano-4- or -6-substituted pyridines (**8**) (Scheme I). After this work was completed, both 1,1,1-trifluoro-2,4-pentanedione[19] and ethyl 4,4,4-trifluoroacetoacetate[19] were reported to react with cyanoacetamide to give, in both cases, the expected 4-trifluoromethyl-2(1H)-pyridones, namely, 3-cyano-4-trifluoromethyl-6-methyl-2(1H)-pyridone[20,21] and 3-cyano-4-trifluoromethyl-6-hydroxy-2(1H)-pyridone,[21] respectively.

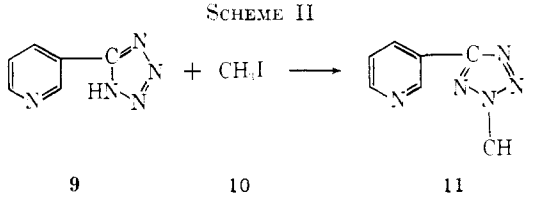
SCHEME I



The potassium permanganate oxidation of 5-[3-(5-methylpyridyl)]tetrazole gave 3-(5-tetrazolyl)pyridine-5-carboxylic acid, an analog of nicotinic acid having the acidic tetrazole function at the 5 position. Treatment of 5-(3-pyridyl)tetrazole with 30% hydrogen peroxide in glacial acetic acid led to 3-(5-tetrazolyl)pyridine N-oxide.

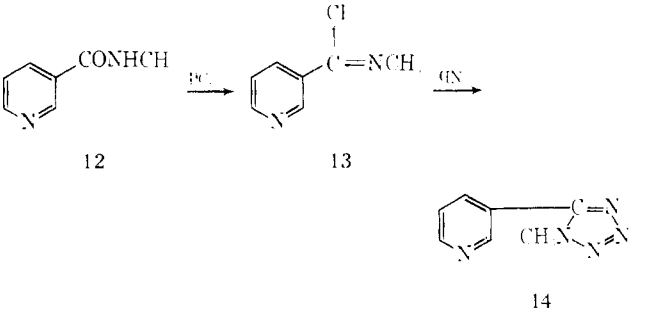
Alkylation of a 5-substituted tetrazole has already been demonstrated to give mainly the 2-alkyl-5-substituted tetrazole.[22,23] Treatment of 5-(3-pyridyl)-tetrazole (**9**) with methyl iodide (**10**) gave a product which we have designated as 2-methyl-5-(3-pyridyl)-tetrazole (**11**) (Scheme II). The 1-position isomer of

SCHEME II



11, namely, 1-methyl-5-(3-pyridyl)tetrazole (**14**), was prepared by a procedure useful for the synthesis of 1-alkyl-5-substituted tetrazoles[24] (Scheme III). The pyridyl-substituted 5-(3-pyridyl)tetrazoles and the 5-heterocyclic-substituted tetrazoles are listed in Tables I and II, respectively.

SCHEME III



Results and Discussion

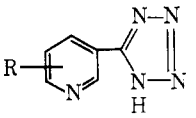
Inhibition of FFA Release from Isolated Adipose Tissue.—The inhibition of the norepinephrine-induced release of FFA from isolated rat adipose tissue by the pyridyl- (Table I) and other heterocyclic (Table II) tetrazoles is expressed as the molar concentration required to produce 50% inhibition (IC_{50}). These activities are listed in Table III. Also included are some close structural analogs of nicotinic acid, including picolinic and isonicotinic acids, nicotinamide, 3-pyridylacetic acid, and the pyridinedicarboxylic acids. Nicotinic acid was the most active, a concentration of only 2×10^{-7} *M* produced 50% inhibition. 3-Pyridylacetic acid, a known hypocholesterolemic agent, also exhibited high activity. Both picolinic and isonicotinic acid were only very weakly active. It is worth noting that the metabolites of nicotinic acid in man, namely, nicotinamide, nicotinuric acid, and N′-methylnicotinamide ($IC_{50} > 10^{-3}$ *M*), were found to be approximately 10,000 times less effective as lipolysis inhibitors than nicotinic acid. The nexus between lipolysis inhibition and lipid lowering is further strengthened by the observation that nicotinic acid and 3-pyridylacetic acid are potent lipolysis inhibitors, whereas nicotinamide is essentially inactive both as a lipolysis inhibitor and as a hypocholesteremic agent.[9]

Of the pyridyl-substituted 5-(3-pyridyl)tetrazoles (Table I) only the parent (R = H) and the 5-CH_3 analog (R = 5-CH_3) showed high activity. Both, however, are about 3000 times less potent than nicotinic acid. It can be concluded, therefore, that the replacement of the carboxyl function in nicotinic acid by the tetrazole moiety causes a substantial decrease in intrinsic lipolysis inhibitory activity. Substituents on the pyridine nucleus, whether electron donating or electron attracting, are on the whole detrimental to *in vitro* lipolysis inhibitory activity. The 5-heterocyclic substituted tetrazoles (Table II) were consistently much less potent than nicotinic acid. Like the pyridinecarboxylic acids, location of the acidic tetrazole function at either the 2 or 4 position on the pyridine nucleus, namely, 5-(2-pyridyl)tetrazole and 5-(4-pyridyl)tetrazole, decreased *in vitro* activity. Replacement of the pyridyl group by the pyrazinyl, pyrimidinyl, quinolyl, and isoxazolyl moieties substantially decreased *in vitro* activity. The tetrazole analog of 3-pyridylacetic acid

(19) Columbia Organic Chemicals Co., Inc., Columbia, S. C.
(20) J. L. Greene, Jr., and J. A. Montgomery, *J. Med. Chem.*, **6**, 294 (1963).
(21) S. Portnoy, *J. Org. Chem.*, **30**, 3377 (1965).
(22) B. Elpern and F. C. Nachod, *J. Am. Chem. Soc.*, **72**, 3379 (1950).
(23) R. A. Henry, *ibid.*, **73**, 4470 (1951).
(24) B. Elpern, *ibid.*, **75**, 661 (1953).

TABLE I

PYRIDYL-SUBSTITUTED 5-(3-PYRIDYL)TETRAZOLES

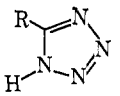


R	Mp, °C	Formula	% calcd			% found		
			C	H	N	C	H	N
H[a]	238 dec	$C_6H_5N_5$	48.97	3.43	47.60	48.84	3.40	47.62
4-CH_3	225–227 dec	$C_7H_7N_5$	52.16	4.38	43.46	51.86	4.44	43.24
5-CH_3	223	$C_7H_7N_5$	52.16	4.38	43.46	52.06	4.52	43.28
6-CH_3	228–230 dec	$C_7H_7N_5$	52.16	4.38	43.46	52.19	4.56	43.59
4-CF_3	183–186.5	$C_7H_4F_3N_5$	39.08	1.87	32.55	38.98	2.08	32.32
5-F	204 dec	$C_6H_4FN_5$	43.63	2.44	42.41	43.31	2.53	42.66
2-OCH_3	158–160 dec	$C_7H_7N_5O$	47.45	3.98	39.53	47.58	4.08	39.38
6-OCH_3	204 dec	$C_7H_7N_5O$	47.45	3.98	39.53	47.27	4.06	39.22
2-SCH_3	213–214 dec	$C_7H_7N_5S$	43.52	3.65	36.26	43.55	3.65	36.14
6-SCH_3	214–215 dec	$C_7H_7N_5S$	43.52	3.65	36.26	43.40	3.71	36.06
6-SO_2CH_3	199–200 dec	$C_7H_7N_5O_2S$	37.34	3.13	31.11	37.56	3.39	31.28
5-NH_2	322 dec	$C_6H_6N_6$	44.44	3.74	51.82	44.45	3.94	51.64
6-NH_2	309 dec	$C_6H_6N_6$	44.44	3.74	51.82	44.23	4.01	50.74
6-$NHCOCH_3$	281 dec	$C_8H_8N_6O$	47.05	3.95	41.16	47.27	4.24	41.21
5-COOH	284–285 dec	$C_7H_5N_5O_2$	43.98	2.64	36.64	44.16	2.89	36.94
2-OH-6-CH_3	310–312 dec	$C_7H_7N_5O$	47.45	3.98	39.53	47.46	4.09	39.80
4,6-$(CH_3)_2$	231–233 dec	$C_8H_9N_5 \cdot HCl$	45.40	4.76	33.09	45.58	4.95	33.24
4-CF_3-6-CH_3	204 dec	$C_8H_6F_3N_5 \cdot HCl$	36.17	2.66	26.37	36.42	2.71	25.83
4-CH_2OCH_3-6-CH_3	204–205 dec	$C_9H_{11}N_5O \cdot HCl$	44.72	5.00	28.98	44.86	5.09	28.93

[a] W. J. van der Burg, *Rec. Trav. Chim.*, **74**, 257 (1955).

TABLE II

5-HETEROCYCLIC-SUBSTITUTED TETRAZOLES



R	Mp, °C	Formula	% calcd			% found		
			C	H	N	C	H	N
2-Pyridyl[a]	215–216 dec							
4-Pyridyl[a]	263 dec							
2-Pyrazinyl[b]	180–182							
2-Pyrimidinyl[c]	229–230 dec	$C_5H_4N_6$	40.54	2.72	56.74	40.57	2.71	57.12
3-Quinolyl	249–251 dec	$C_{10}H_7N_5$	60.90	3.58	35.52	60.58	3.80	35.28
3-Pyridyl N-oxide	248 dec	$C_6H_5N_5O$	44.17	3.09	42.93	44.00	3.17	42.93
3-Pyridylmethyl	192–193 dec	$C_7H_7N_5 \cdot HCl$	42.54	4.08	35.44	42.54	4.19	35.42
3-(5-Methyl)isoxazolyl	170–171.5	$C_5H_5N_5O$	39.73	3.33	46.34	39.79	3.53	46.20
5-(3-Methyl)isoxazolyl	188.5–190	$C_5H_5N_5O$	39.73	3.33	46.34	39.92	3.40	46.16
1-Methyl-5-(3-pyridyl)tetrazole	78–80	$C_7H_7N_5$	52.16	4.38	43.46	52.12	4.41	43.08
2-Methyl-5-(3-pyridyl)tetrazole	127.5–129	$C_7H_7N_5$	52.16	4.38	43.46	52.13	4.42	43.58

[a] Reference *a*, Table I. [b] S. Kushner, H. Dalalian, J. L. Sanjurjo, F. L. Bach, Jr., S. R. Safir, V. K. Smith, Jr., and J. H. Williams, *J. Am. Chem. Soc.*, **74**, 3617 (1952). [c] M. Robba, *Ann. Chim.* (Paris), **5**, 351 (1960).

was inactive. Lastly, removing the acidic character[25] of the active 5-(3-pyridyl)tetrazole (**9**) by methylation at either the 2 or 1 position of the tetrazole function, **11** and **14**, respectively, abolished antilipolytic activity. In summary, the structural requirements within the tetrazole family for high lipolysis inhibitory activity in isolated adipose tissue consist of an acidic tetrazole group located at the 3 position on pyridine.

Effect on Fasting Plasma FFA.—The effects of the pyridylcarboxylic acids and pyridyl- and other heterocyclic tetrazoles on the fasting plasma FFA levels of the dog are described in Table IV. Of the pyridinecarboxylic acids evaluated, only nicotinic acid and 3-pyridylacetic acid produced a maximal reduction (>60%) of plasma FFA.

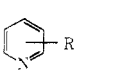
The structural requirements for *in vivo* lipolysis inhibitory activity among the pyridyl- and other heterocyclic tetrazoles were found to be quite restrictive. Within the pyridyl-substituted 5-(3-pyridyl)tetrazole series (Table I), 5-(3-pyridyl)tetrazole (R = H) was the most active. It gave a maximal reduction of plasma FFA in the dog, like nicotinic acid, with a dose of 10 mg/kg. A number of other members of this series (R = 5-CH_3, 5-F, 5-NH_2, 6-$NHCOCH_3$, 5-COOH, and 2-SCH_3) were moderately active and produced less than a maximal reduction (30–60%) of plasma FFA.

The 2-pyrazinyl, 3-pyridylmethyl, and 3-pyridyl N-oxide members of the 5-(heterocyclic)tetrazole series (Table II) had modest FFA depressing activity. They gave less than a maximal reduction of plasma FFA (30–60%). No member of this series produced a

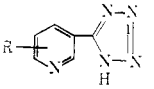
(25) The apparent ionization constant of 5-(3-pyridyl)tetrazole (pK_a = 4.1) was quite similar to the apparent ionization constant of nicotinic acid (pK_a = 4.5). They were determined by potentiometric titrations, using a Beckman Model G pH meter, in ethanol–H_2O (50%, v/v) medium with standard 0.5 *N* NaOH. The apparent pK_a values correspond to the pH at the 50% neutralization point in these titration curves.

TABLE III

INHIBITION OF NOREPINEPHRINE-INDUCED FFA RELEASE FROM ISOLATED ADIPOSE TISSUE



R	IC_{50}, M[a]
3-COOH	2×10^{-7}
3-CH_2COOH	6×10^{-5}
2,3-(COOH)$_2$	8×10^{-4}
2,6-(COOH)$_2$	6×10^{-4}
2-COOH	10^{-3}
2,4-(COOH)$_2$	10^{-3}
3-CONHCH$_2$COOH	10^{-3}
4-COOH	$>10^{-3}$
3-$CONH_2$	$>10^{-3}$
2,5-(COOH)$_2$	$>10^{-3}$
3,5-(COOH)$_2$	$>10^{-3}$



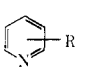
R	IC_{50}, M[a]
H	6×10^{-4}
5-CH_3	6×10^{-4}
4-CH_3	10^{-3}
5-NH_2	$>10^{-3}$
6-NH_2	$>10^{-3}$
5-COOH	$>10^{-3}$
4,6-(CH$_3$)$_2$	$>10^{-3}$
2-SCH_3	$>10^{-3}$
6-CH_3	$>10^{-3}$
4-CF_3	$>10^{-3}$
2-OCH_3	Inactive
6-OCH_3	Inactive
6-SCH_3	Inactive
2-OH-6-CH_3	Inactive

R	IC_{50}, M[a]
2-Pyridyl	10^{-3}
3-Pyridyl N-oxide	10^{-3}
5-(3-Methyl)isoxazolyl	$>10^{-3}$
2-Pyrazinyl	$>10^{-3}$
3-Pyridylmethyl	$>10^{-3}$
4-Pyridyl	Inactive
2-Pyrimidinyl	Inactive
3-Quinolyl	Inactive
1-Methyl-5-(3-pyridyl)tetrazole	Inactive
2-Methyl-5-(3-pyridyl)tetrazole	Inactive

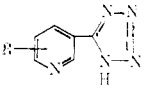
[a] The molar concentration required to produce 50% inhibition of the norepinephrine-induced FFA release from adipose tissue *in vitro*.

TABLE IV

EFFECT OF PYRIDYLCARBOXYLIC ACIDS, PYRIDYLTETRAZOLES, AND HETEROCYCLIC TETRAZOLES ON FASTING PLASMA FFA *in Vivo*[a]



R	Activity[b]
3-COOH	++
3-CH_2COOH	−+
4-COOH	±
3-CONHCH$_2$COOH	±
2,3-(COOH)$_2$	±
2,6-(COOH)$_2$	±



R	Activity[b]
H	−+
5-CH_3	+
5-F	+
5-NH_2	−
6-$NHCOCH_3$	+
5-COOH	+
2-SCH_3	+
4-CH_3	±
6-CH_3	±
4-CF_3	±
6-CH_3SO_2	±
6-NH_2	±
4-CF_3-6-CH_3	±
4-CH_2OCH_3-6-CH_3	±

R	Activity[b]
2-Pyrazinyl	+
3-Pyridylmethyl	+
3-Pyridyl N-oxide	+
2-Pyridyl	±
4-Pyridyl	±
2-Pyrimidinyl	±
3-Quinolyl	±
5-(3-Methyl)isoxazolyl	±
3-(5-Methyl)isoxazolyl	±
1-Methyl-5-(3-pyridyl)tetrazole	±

[b] In the dog, 10 mg/kg iv. ++, maximal reduction of plasma FFA (>60%); −, less than maximal reduction of plasma FFA (30–60%); ±, inactive or less than 30% reduction of plasma FFA.

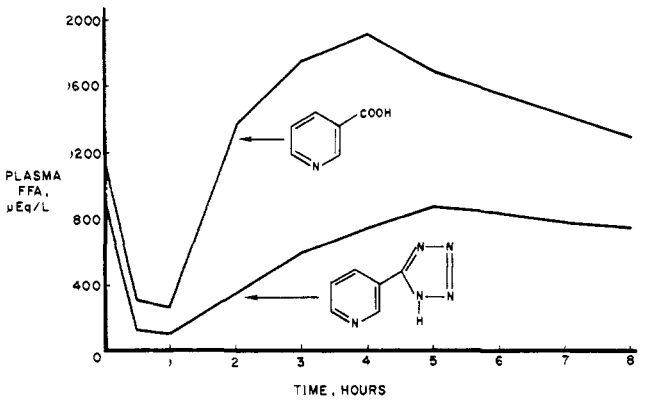


Figure 1.—Time course of the effects of nicotinic acid and 5-(3-pyridyl)tetrazole on fasting plasma FFA in the dog, 10 mg/kg iv.

maximal reduction of plasma FFA. Similar to the findings obtained in the *in vitro* testing procedure, location of the acidic tetrazole function at either the 2 or 4 position on the pyridine nucleus, namely, 5-(2-pyridyl)tetrazole and 5-(4-pyridyl)tetrazole, markedly decreased *in vivo* activity. As expected, removing the acidic character of 5-(3-pyridyl)tetrazole by replacing the acidic tetrazole hydrogen by a methyl group (**14**) abolished activity.

The similar intensities in plasma FFA depression exhibited by both 5-(3-pyridyl)tetrazole (**9**) and nicotinic acid were interesting in view of the modest activity of 5-(3-pyridyl)tetrazole in the isolated adipose tissue system. A comparison of the duration of action in the dog between both these acidic compounds was made after the administration of 10 mg kg. A dose of 10 mg/kg of nicotinic acid produced a maximal reduction of plasma FFA between 0.5 and 1 hr following intravenous administration (Figure 1). The plasma FFA returned to the control level after approximately 2 hr and then rose above this level for the remainder of the 8-hr observation period. A similar dose of 5-(3-pyridyl)tetrazole also caused a maximal reduction of plasma FFA between 0.5 and 1 hr. However, the duration of plasma FFA depression was much longer, approximately 5 hr, with 5-(3-pyridyl)tetrazole (Figure 1).

The improved duration of action of 5-(3-pyridyl)tetrazole over nicotinic acid, in spite of a decreased activity in the isolated adipose tissue system, is probably attributable to its greater metabolic stability.[26]

The extended duration of FFA-depressing activity of 5-(3-pyridyl)tetrazole in the dog prompted a clinical evaluation of this compound. Preliminary results show that it lowers plasma FFA and cholesterol on repeated administration to man.[27] A more detailed analysis of the pharmacological and clinical profiles of 5-(3-pyridyl)tetrazole will be published elsewhere.

Experimental Section[28]

5-(3-Pyridyl)tetrazole.—A stirred mixture of 1500 ml of dry (molecular sieve) DMF, 234 g (2.24 moles) of 3-cyanopyridine, 195 g (3 moles) of NaN_3, 162 g (3 moles) of NH_4Cl, and 3 g o LiCl was heated to 125° for 12 hr. The insolubles, after cooling to room temperature, were removed by suction filtration, and the DMF was distilled *in vacuo*. The crude product remaining after the solvent was removed was dissolved in 4 l. of water and the pH was adjusted to 4 with HCl. There was obtained 166 g of product, mp 234° dec. Adjusting the aqueous filtrate to pH 2 gave an additional 20 g, mp 234° dec. Recrystallization of both crops from water led to 160 g (49% yield) of purified product, mp 238° dec.

All the pyridyl- and heterocyclic tetrazoles prepared from the corresponding cyano compounds were made by essentially the same procedure.

3-Cyanopyridines.—The following 3-cyanopyridines were available or prepared by literature procedures: 3-cyanopyridine,[29] 3-cyano-4-methylpyridine,[30] 3-cyano-5-methylpyridine,[29] 2-methoxy-3-cyanopyridine,[31] 3-cyano-6-methoxypyridine,[32] 3-cyano-6-methylthiopyridine,[32] 3-cyano-6-methylsulfonylpyridine,[32] 3-cyano-6-aminopyridine,[33] 3-cyano-6-acetamidopyridine,[34] 3-cyano-4,6-dimethylpyridine,[35] 3-cyano-6-methyl-2(1H)-pyridone,[36] 3-cyanoquinoline,[37] 3-cyano-5-methylisoxazole,[38] and 3-methyl-5-cyanoisoxazole.[38]

3-Cyano-6-methylpyridine.—A mixture of 14.6 g (0.096 mole) of 2-chloro-3-cyano-6-methylpyridine[39] and 14.2 ml (0.106 mole) of triethylamine in 400 ml of methanol containing 2 g of 5% Pd–C was shaken under 2.8 kg of hydrogen/cm² at 25°. After 1 hr the theoretical amount of hydrogen was absorbed, the suspension was filtered, and the residue was washed well with methanol. The solvent was removed *in vacuo*, 100 ml of water was added, and after filtering there was obtained 5.3 g of product, mp 80–81°.[39] The aqueous filtrate was saturated with NaCl and extracted with ether. An additional 2.3 g, mp 80–81°, was obtained by removing the ether *in vacuo*. The total yield was 7.6 g (65%).

3-Cyano-4-trifluoromethylpyridine.—To a mixture of 90 g (1.07 moles) of cyanoacetamide and 91 g (1.07 moles) of piperidine in 700 ml of absolute ethanol at 70° was added dropwise 178 g (1.04 moles) of ethyl 4,4,4-trifluoroacetoacetate.[19] After heating to reflux for 12 hr, the mixture was cooled and filtered. The residue was dissolved in 2 l. of water, and the solution was acidified with dilute HCl. After filtering, the crude 3-cyano-4-trifluoromethyl-6-hydroxy-2(1H)-pyridone was recrystallized from 1 l. of water to give 165 g (83% yield), mp 192–195.5°.[40] This material was not purified further but treated with $POCl_3$, under the same conditions used for the preparation of 3-cyano-2,6-dichloro-4-methylpyridine,[30] to give 3-cyano-2,6-dichloro-4-trifluoromethylpyridine, 25 g (63% yield), bp 68–71° (0.5 mm), mp 39–40°.

Anal. Calcd for $C_7HCl_2F_3N_2$: C, 34.88; H, 0.42; N, 11.62. Found: C, 34.99; H, 0.46; N, 11.20.

Dehalogenation by the same procedure used for the preparation of 3-cyano-6-methylpyridine gave 3-cyano-4-trifluoromethylpyridine in 50% yield, bp 70° (0.07 mm).

Anal. Calcd for $C_7H_3F_3N_2$: C, 48.84; H, 1.75; N, 16.27. Found: C, 48.72; H, 2.04; N, 15.91.

3-Cyano-5-fluoropyridine.—A mixture of 2.5 g of 5-fluoronicotinamide[41] and 5 g of P_2O_5 were intimately mixed and heated at 230° under 0.01 mm of pressure. During a period of 1 hr 1.39 g (64% yield) of product distilled, mp 54–55.5°.

Anal. Calcd for $C_6H_3FN_2$: C, 59.02; H, 2.48; N, 22.95. Found: C, 58.81; H, 2.26; N, 23.05.

2-Methylthio-3-cyanopyridine.—The same sequence of reactions used in the preparation of 3-cyano-6-methylthiopyridine,[32] but starting with 2-chloro-3-cyanopyridine,[42] was carried out, mp 87.5–89.5°.

Anal. Calcd for $C_7H_6N_2S$: C, 56.00; H, 4.03; N, 18.66. Found: C, 55.79; H, 3.82; N, 18.53.

3-Cyano-5-aminopyridine.—A solution of 45 g of $SnCl_2 \cdot 2H_2O$ in 90 ml of concentrated HCl was added to 50 ml of ether containing 10 g (0.054 mole) of 2-chloro-3-cyano-5-nitropyridine.[43] The initial reaction was exothermic; the mixture was stirred vigorously until the temperature had fallen to 30°, diluted with 200 ml of water, made strongly basic with 40% NaOH, cooled, and filtered. There was obtained 7.9 g (93% yield) of 2-chloro-3-cyano-5-aminopyridine, mp 192.5–194.° An analytical sample was prepared by a recrystallization from methanol, mp 193.5–194°.

Anal. Calcd for $C_6H_4ClN_3$: C, 46.92; H, 2.63; N, 27.36. Found: C, 46.65; H, 2.64; N, 27.00.

Dehalogenation by the same procedure used for the preparation of 3-cyano-6-methylpyridine gave 3-cyano-5-aminopyridine, 3.3 g (55% yield), mp 118–123°. An analytical sample was prepared by a recrystallization from chloroform–hexane, mp 125–127.5°.

Anal. Calcd for $C_6H_5N_3$: C, 60.49; H, 4.23; N, 35.28. Found: C, 60.53; H, 3.93; N, 35.52.

3-(5-Tetrazolyl)pyridine-5-carboxylic Acid.—Over a period of 6 hr, 175 ml of a warm aqueous solution of 1 *M* $KMnO_4$ was added dropwise to a solution, maintained at 90–100°, of 4 g (0.025 mole) of 5-[3-(5-methylpyridyl)]tetrazole in 150 ml of water. This mixture was refluxed for an additional 16 hr. The filtrate, after cooling and filtering, was concentrated *in vacuo* to a volume of about 50 ml. The product separated after acidification to pH 3–4 with HCl. After washing with water there was obtained 1.1 g (24% yield) of product, mp 279–283° dec. Two recrystallizations from methanol–ether raised the melting point to 284–285° dec.

3-Cyano-4-trifluoromethyl-6-methylpyridine.—To a mixture of 90 g (1.07 moles) of cyanoacetamide and 14 ml (0.14 mole) of piperidine in 750 ml of absolute ethanol at 75° was added dropwise 150 g (0.97 mole) of 1,1,1-trifluoro-2,4-pentanedione.[19] After heating to reflux for 3 hr, 750 ml of water was added and the product, after cooling, was collected by suction filtration, 147 g, mp 231–233°.[20,21] An additional 19 g was obtained from the mother liquor after acidification with acetic acid. The total yield of 3-cyano-4-trifluoromethyl-6-methyl-2(1H)-pyridone was 85%, and this material was used in the next step without further purification. A mixture of 27.9 g (0.136 mole) of the pyridone, 31.2 g (0.15 mole) of PCl_5, and 125 ml of $POCl_3$ was heated under gentle reflux for 18 hr. After cooling, 60 ml of toluene was added, and the mixture was concentrated *in vacuo* to constant weight. The residue was cooled and 30 ml of ethanol

(26) Dr. M. Schach von Wittenau of these laboratories has shown that 5-(3-pyridyl)tetrazole is excreted by the dog essentially unchanged over a 24-hr period.

(27) Unpublished observations of Drs. S. Gilgore and S. DeFelice of our Clinical Pharmacology Department.

(28) Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Boiling points are uncorrected. The analyses were carried out by the Physical Measurements Laboratory of Chas. Pfizer & Co., Inc.

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and 100 ml of water was added. An oil separated which was extracted into ether. Removal of the ether followed by distillation gave 23.9 g (80% yield) of 2-chloro-3-cyano-4-trifluoromethyl-6-methylpyridine, bp 85° (0.05 mm).

Anal. Calcd for $C_8H_4ClF_3N_2$: C, 43.55; H, 1.82; N, 12.69. Found: C, 43.19; H, 2.12; N, 12.24.

Dehalogenation by the same procedure used for the preparation of 3-cyano-6-methylpyridine gave 3-cyano-4-trifluoromethyl-6-methylpyridine in 52% yield, bp 50–52° (0.02 mm).

Anal. Calcd for $C_8H_5F_3N_2$: C, 51.61; H, 2.70; N, 15.04. Found: C, 51.54; H, 2.75; N, 15.06.

3-Cyano-4-methoxymethyl-6-methylpyridine.—Dehalogenation of 2-chloro-3-cyano-4-methoxymethyl-6-methylpyridine[43] by the same procedure used for the preparation of 3-cyano-6-methylpyridine gave the product in 66% yield, bp 150–155° (0.06–0.08 mm), mp 44–45°.

Anal. Calcd for $C_9H_{10}N_2O$: C, 66.65; H, 6.22; N, 17.27. Found: C, 66.29; H, 6.14; N, 17.30.

3-(5-Tetrazolyl)pyridine N-Oxide.—A solution containing 14.7 g (0.1 mole) of 5-(3-pyridyl)tetrazole, 75 ml of acetic acid, and 225 ml of 30% H_2O_2 was maintained at 90° for 24 hr. On cooling and triturating with ether there was obtained 11.1 g of crude product, mp 235° dec. Recrystallization from water gave 6.0 g (37% yield) of purified product, mp 248° dec.

5-(3-Pyridylmethyl)tetrazole Hydrochloride.—A mixture of 20 g of acetic acid, 26 g (0.22 mole) of 3-pyridylacetonitrile,[45] 100 ml of *n*-butyl alcohol, and 22 g (0.33 mole) of NaN_3 was heated to reflux for 4 days. The mixture was cooled and an additional 5 g of NaN_3 and 10 g of acetic acid was added and heating under reflux was continued for 2 additional days. After cooling, 300 ml of water was added and the *n*-butyl alcohol was removed *in vacuo*. Purification was achieved by way of the copper tetrazole derivative. Addition of a solution of 21.9 g of copper acetate in 200 ml of water precipitated the copper salt. After washing with water, the salt was suspended in 400 ml of water and H_2S was bubbled in until the precipitation of CuS was complete. The clear aqueous filtrate, after removing the CuS, was concentrated to dryness *in vacuo*. There was obtained 15.8 g of crude 5-(3-pyridylmethyl)tetrazole. Addition of a solution of ethyl acetate saturated with dry HCl to an ethanol solution of this product gave the salt, 17.5 g (40% yield), mp 188–190° dec. An analytical sample was prepared by a recrystallization from methanol–ether, mp 192–193° dec.

Anal. Calcd for $C_7H_8ClN_5$: C, 42.54; H, 4.08; N, 35.44; Cl, 17.94. Found: C, 42.54; H, 4.19; N, 35.42; Cl, 17.96.

1-Methyl-5-(3-pyridyl)tetrazole.—A mixture of 6.8 g (0.05 mole) of N-methylnicotinamide[45] and 10.4 g (0.05 mole) of PCl_5 in 125 ml of benzene was stirred at 25° for 6.5 hr. After this time, 30 ml of a solution of 4 *N* hydrazoic acid in benzene was added and stirring at 25° was continued for an additional 12 hr. After decanting the benzene from the insoluble gum that had formed, 50 ml of a dilute aqueous NaOH solution was added, and the product was extracted (CH_2Cl_2). Removal of the methylene chloride *in vacuo* gave 6.1 g of crude product, mp 66–72°. Recrystallizations from CH_2Cl_2–ether and from toluene gave 2.9 g (36% yield) of purified product, mp 78–80°. The analytical sample was prepared by sublimation, mp 78–80°.

2-Methyl-5-(3-pyridyl)tetrazole.—A suspension of 2.94 g (0.02 mole) of 5-(3-pyridyl)tetrazole and 1.23 ml (0.02 mole) of CH_3I in 40 ml of acetone was treated with a solution of 2.4 g (0.06 mole) of NaOH in 4 ml of water. The mixture was stirred and heated under reflux for 3 hr, filtered, diluted with 20 ml of water, and extracted with benzene. The organic layer was dried and concentrated *in vacuo*. Addition of 25 ml of water to the residue gave 0.97 g (30% yield) of product, mp 127–130°. The analytical sample was prepared by sublimation, mp 127.5–129°.

Pharmacology. Inhibition of FFA Release from Isolated Adipose Tissue.—The inhibition of norepinephrine-induced release of fatty acids was studied with epididymal adipose tissue taken from male Sprague–Dawley rats, 180–240 g, fed *ad libitum*. The tissue was placed in freshly aerated Krebs–Ringer bicarbonate buffer, pH 7.4, and minced with scissors into pieces weighing approximately 10 mg. Each experimental flask contained 3 ml of freshly aerated (95% O_2–5% CO_2) Krebs–Ringer bicarbonate buffer and 200 ± 3 mg (mean ± standard deviation) of adipose tissue. Bovine plasma albumin, fraction IV, 1%, was used as a fatty acid acceptor in the incubation medium. Adequate norepinephrine (20–30 ng/ml) was added to the incubation mixture to elicit a 50% of maximum fatty acid release. The compounds under test were added at appropriate concentrations. The experimental flasks were stoppered, aerated with 95% O_2–5% CO_2 for 10 min and incubated at 37° for 3 hr on a Dubnoff metabolic shaker. After incubation, aliquots were removed for fatty acid analysis by the method of Dole.[46] The effects of the inhibitors were expressed in terms of the molar concentration required to produce 50% inhibition (IC_{50}).

Effect on Fasting Plasma FFA.—An intravenous dose of 10 mg/kg of test compound was administered to two or more normal, fasted dogs. Blood samples were withdrawn for controls and at 0.5, 1 hr, and hourly through 8 hr. Plasma FFA levels were measured by the method of Dole[46] and are expressed as microequivalents of FFA per liter of plasma. A depression of plasma FFA with an intensity greater than 60% is classified as a maximal reduction (++), a 30–60% depression is classified as a less than maximal reduction (+), and those compounds which produce less than a 30% fall of plasma FFA or are inactive are grouped together (±).

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The Antifertility Activity of Isoflavones Related to Genistein

G. W. MOERSCH, D. F. MORROW, AND W. A. NEUKLIS

Parke, Davis and Company, Research Laboratories, Ann Arbor, Michigan

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A group of 35 isoflavones has been synthesized by known procedures. They were tested for antifertility effects in a mouse litter prevention assay and as hypocholesteremic agents in normal rats.

Although estrogens alone are effective antifertility agents,[1] they are employed either in combination with a progestin or sequentially, *i.e.*, followed by a progestin. The chronic administration of small doses of estrogen delays bleeding until 7–10 days following cessation of therapy, while chronic administration of larger doses of estrogen leads to irregular bleeding or spotting due to endometrial hyperplasia.[2] It has recently been re-

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