Journal of Natural Products
Vol. 51, No. 5, pp. 862-865, Sep-Oct 1988

INHIBITION OF DOPAMINE β-HYDROXYLASE BY GOITRIN, A NATURAL ANTITHYROID COMPOUND

NICOLAS ZENKER,* LOU S. HUBBARD, and JEREMY WRIGHT

Department of Medicinal Chemistry/Pharmacognosy, School of Pharmacy, University of Maryland at Baltimore, 20 North Pine Street, Baltimore, Maryland 21201

ABSTRACT.—*RS*-Goitrin can be conveniently prepared by a simplification of the Ettlinger procedure. Goitrin is a moderate inhibitor of purified bovine adrenal dopamine β-hydroxylase. The administration of goitrin leads to a depression of brain norepinephrine and to an elevation of heart and adrenal dopamine.

Goitrin is a compound present in cabbage, rutabaga, turnip, and brassicaceous weeds (1,2). Goitrin is an antithyroid agent that is equally active in its enantiomeric or racemic forms (3); it has been described as more potent than propylthiouracil in man (2,3) and has caused outbreaks of endemic goiter (2) when milk from cows feeding on goitrin-containing brassicaceous weeds was ingested. Chemically, goitrin (*R*-5-vinyl-2-thiooxazolidone) has in its structure a thioamide group that should enable it to chelate cupric ions (4,5) and, therefore, to inhibit copper-requiring enzymes such as dopamine-β-hydroxylase (DBH). Because goitrin is occasionally ingested in substantial quantities and since other thioamide-containing compounds such as the antithyroid agents propylthiouracil (PTU) and methimazole (6) have been shown to have DBH inhibitory activity, we investigated the effects of goitrin on DBH and on tissue catecholamines.

EXPERIMENTAL

PREPARATION OF *RS*-GOITRIN.—Epoxybutene (butadiene monoxide) was obtained from Aldrich. The ammonolysis of epoxybutene (Scheme 1) yielded 1-amino-3-buten-2-ol, which was isolated as the acid oxalate salt following the procedure of Ettlinger (7). The aminobutenol oxalate (17.8 g, 0.1 mol) was directly reacted with aqueous KOH (0.3 mol) and carbon disulfide (0.1 mol) and the Ettlinger procedure followed in all other details; this simplified preparation is presented in Scheme 1. After two recrystallizations from Et_2O, 3.7 gm (29%) of *RS*-goitrin was obtained.

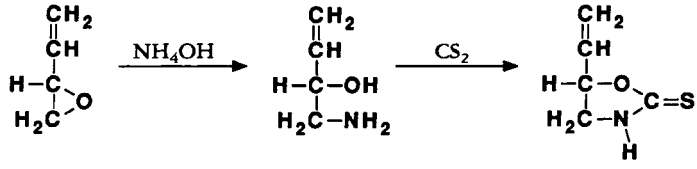
RS-Goitrin was characterized by melting point (Thomas-Hoover, uncorrected), by uv (Gilford 2600), nmr (GE QE 300), and ms (Extrel EL-1000) as well as by elemental analysis (Galbraith Labs., Kentucky).

RS-Goitrin melted at 62.5–64° [lit. (7) 64–65°]; its uv max (H_2O) was at 240.5 nm, log ε = 4.20 [lit. (8) max 240 nm, log ε = 4.18).

The ^{1}H-nmr spectrum showed multiplets at δ 6.0 (vinyl methine), 5.37 (vinyl methylene), 3.85 (C-5 methane), and 3.37 (C-4 methylene). The ^{13}C-nmr spectrum (off-resonance decoupled) gave signals at 189 ppm (s, thione), 135 (d, vinyl methine), 121 (t, vinyl methylene), 83 (d, C-5), and 44 (t, C-4).

The mass spectral peaks (main fragmentation peaks 39, 54, 68, 85, *m/z* 102, 129) were consistent with those previously reported (9). ^{1}H-nmr, ^{13}C-nmr, and ms peaks were identical to those of a sample of *R*-goitrin isolated from *Crambe abyssinica* and generously provided by Drs. Daxenbichler and Nishie of the Department of Agriculture.

Elemental analysis for *RS*-goitrin, C_5H_7NOS: calcd C 46.49, H 5.46, N 10.84, S 24.82, found C 46.52, H 5.50, N 10.73, S 24.94.



SCHEME 1

BIOCHEMICAL ASSAYS.—DBH was purified according to the procedure of Foldes *et al.* (10) from bovine adrenal medullae obtained from the Pel-Freeze Company. All biochemical reagents were obtained from the Sigma Chemical Company. DBH activity was measured in triplicate samples using the incubation conditions described by Matsui *et al.* (11) with dopamine (DA) serving as substrate; the reaction was terminated by the addition of an aliquot of incubation mixture to an equal volume of ice-cold 1N $HClO_4$. The supernatant was then processed for catecholamine analysis as described by Refshauge *et al.* (12), and the norepinephrine (NE) produced was analyzed by liquid chromatography followed by electrochemical detection as described by Felice *et al.* (13), with dihydroxybenzylamine (DHBA) used as internal standard and instrumentation obtained from Bioanalytical Systems, Inc., West Lafayette, IN. Protein analyses were performed by the method of Lowry *et al.* (14).

Male Sprague-Dawley rats (Dominion Labs) weighing 121.5 ± 11.0 g were used for assessment of the in vivo effect of goitrin on tissue catecholamines. The animals were sacrificed at set times after the ip administration of 50 mg/kg aqueous *RS*-goitrin or methimazole and the tissues homogenized (12) and prepared for catecholamine analysis as described above. Tissue goitrin was analyzed by the method of Josefsson and Akerstrom (15).

RESULTS AND DISCUSSION

R-Goitrin is either naturally occurring and isolated or synthesized. In the synthetic procedure of Ettlinger (7), the aminobutenol oxalates are resolved, the oxalate removed by $Ba(OH)_2$ treatment, and the free aminobutenols obtained by vacuum distillation. The equal antithyroid activity of racemic goitrin with that of the enantiomeric forms (3) suggested to us a method which would permit the facile synthesis of racemic *RS*-goitrin in two steps.

Using dopamine as a substrate and DBH purified from bovine adrenals, we obtained a K_m of 3.0 mM for dopamine, a value in fair agreement with the value of 1.5 mM obtained by Matsui *et al.* (11) for human cerebrospinal fluid DBH and of 2.0 mM obtained by Sperk *et al.* (16) for the rat adrenal homogenate enzyme.

A preliminary assessment of *RS*-goitrin, presented in Table 1, indicates that *RS*-

TABLE 1. Inhibition of Dopamine-β-Hydroxylase by Goitrin.[a]

Experiment	Inhibitor	NE formed
1	(Control)	220 ± 14
	MMI, 0.2 mM	53 ± 10
	RS-G, 0.2 mM	49 ± 5
2	(Control)	172 ± 2
	RS-G, 0.1 mM	77 ± 3
	R-G, 0.1 mM	62 ± 3

[a]Triplicate incubation mixtures contained 0.2 mmol acetate buffer, pH 5.0, a bovine adrenal, dopamine β-hydroxylase preparation containing 0.36 mg protein, 3 μmol each of *N*-maleimide and pargyline hydrochloride, 30 μmol each of ascorbic acid and sodium fumarate and 10,000 units of catalase in a volume of 950 μl. Fusaric acid, goitrin (*RS*-G or *R*-G), and methimazole (MMI) were dissolved in acetate buffer; the blank tubes contained fusaric acid at 0.125 mM. After 5 min of preincubation at 37°, the reaction was initiated by the addition of 1.0 μmol dopamine in a volume of 50 μl and allowed to proceed for 45 min. The reaction was stopped, and the norepinephrine (NE) formed was measured as described in the Experimental section. Results are expressed as nanomol NE formed per mg of protein in 45 min ± the standard deviation.

goitrin is a moderately effective DBH inhibitor with 0.1 and 0.2 mM *RS*-goitrin leading, respectively, to 55 and 78% inhibition. The data indicate further that goitrin and methimazole are inhibitors of comparable potency. Finally, while the data suggest that *R*-goitrin might be a better inhibitor than the racemic compound, the difference is slight and does not suggest a specific role of the asymmetric C-5.

Increases in adrenal, cardiac, and brain DA and decreases in brain NE following the administration of DBH inhibitors are well documented (17–19). Further, DA increases following the administration of DBH inhibitors have been related to the ability of such inhibitors to prevent the conversion of ^{3}H-DA to ^{3}H-Ne (17), indicating that tissue DA increases and NE decreases were at least in part due to the ability of the inhibitor to produce in vivo DBH inhibition.

Measurements of brain NE concentrations performed at set times after the administration of *RS*-goitrin, methimazole, or vehicle are listed in Table 2. The results in Table

TABLE 2. Effect of *RS*-Goitrin and Methimazole on Rat Brain Norepinephrine Levels.[a]

Time	Control	Methimazole	*RS*-Goitrin
1 h	0.222 ± 0.069	0.216 ± 0.107	0.249 ± 0.090
2.5 h	0.216 ± 0.025	0.143 ± 0.028[b]	0.158 ± 0.038[b]
6 h	0.206 ± 0.022	0.076 ± 0.006[b]	0.092 ± 0.037[b]

[a]Male Sprague-Dawley rats weighing 134 ± 16 g were administered vehicle ip (control) or 50 mg/kg of methimazole or *RS*-goitrin. The animals (5 per group) were sacrificed at set times after the administration of effector or vehicle, and tissue norepinephrine was analyzed by liquid chromatography. The experiment was carried out in 3 days (one for each set time). The norepinephrine concentrations (μg/g) are expressed as the means ± the standard deviation.

[b]$p < 0.001$ vs. control.

2 indicate that goitrin or methimazole had no effect after 1 h but significantly lowered brain NE 2.5 and 6 h after their administration; the results also suggest that goitrin is almost as effective as methimazole in lowering brain NE. In heart and adrenal gland, where the DA concentration is only a small fraction of NE concentration, absolute DA increases are a better index of DBH inhibition. When goitrin (50 mg/kg) or vehicle was administered to groups of 3 rats, there was, 4 h later, an increase from 0.023 ± 0.005 to 0.071 ± 0.005 μg/g ($p<0.001$) in the cardiac DA and from 5.9 ± 1.0 to 10.0 ± 0.9 μg/g ($p<0.01$) in the adrenal DA of the goitrin. At that time the concentration of goitrin, which was 22.3 ± .06 μg/g in the heart and 21.0 ± 1.0 μg/g in the brain, indicated that goitrin had equal access to heart and brain. The effect of goitrin (50 mg/kg) on cardiac catecholamines is illustrated in the liquid chromatography tracings of Figure 1 which were obtained from a pool of control (Figure 1A) and of goitrin-treated rats (Figure 1B) 6 h after the administration of vehicle or goitrin. The tracings, which may be compared by reference to the internal standard DHBA, clearly show the increase in cardiac DA and the decrease in cardiac NE caused by goitrin.

The decrease in brain NE and the increases in adrenal and cardiac DA seen after the administration of goitrin all indicate in vivo DBH inhibition in all three tissues.

That goitrin was nearly as effective as methimazole in lowering brain NE is of interest because methimazole has been claimed to prevent ^{3}H-DA hydroxylations in doses similar to those of fusaric acid (17), a potent in vitro DBH inhibitor. The results we obtained thus suggest that investigating the effects of the consumption of large amounts of goitrin-containing foods on catecholamine levels in man may be of interest.

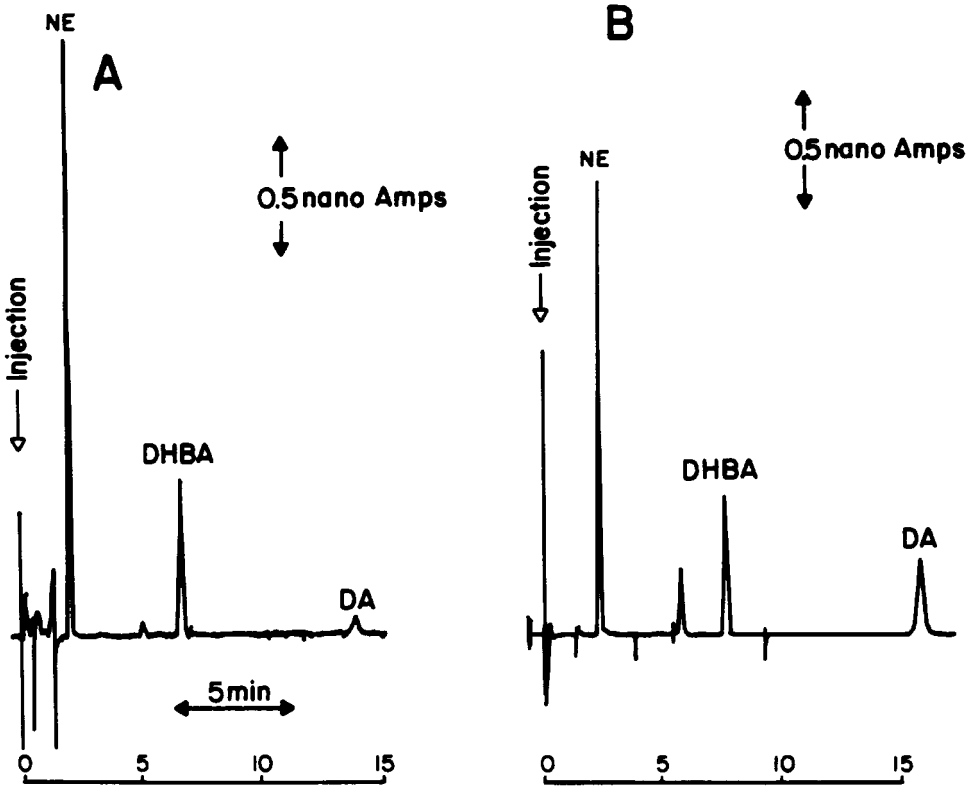


FIGURE 1. Norepinephrine and dopamine from pooled heart homogenates. Liquid chromatography tracings of pools of cardiac homogenates (4 rats/pool) analyzed 6 h after the administration of vehicle (A) or 50 mg/kg goitrin (B). The norepinephrine (NE) and dopamine (DA) of the tracings may be compared by reference to the internal standard dihydroxybenzylamine (DHBA).

LITERATURE CITED

1. A.M. Chesney, T.A. Clawson, and B. Webster, *Johns Hopkins Hosp. Bull.*, **43**, 261 (1928).
2. A. Arstila, F.E. Krusius, and P. Peltola, *Acta Endocr. (Kbh.)*, **60**, 712 (1969).
3. M.A. Greer, *Rec. Progr. Horm. Res.*, **18**, 187 (1962).
4. R.W. Fuller, P.P.K. Ho, C. Matsumoto, and J.A. Clemens, *Adv. Enzyme Reg.*, **15**, 267 (1977).
5. R.C. Rosenberg, *Biochim. Biophys. Acta*, **749**, 276 (1983).
6. H. Hidaka and A. Nagasaka, *Biochem. Pharmacol.*, **26**, 1092 (1977).
7. M.G. Ettlinger, *J. Am. Chem. Soc.*, **72**, 4792 (1950).
8. E.B. Astwood, M.A. Greer, and M.G. Ettlinger, *J. Biol. Chem.*, **181**, 121 (1949).
9. N. Michajlovskij and P. Langer, in: "Further Advances in Thyroid Research." Ed. by K. Fellinger and R. Hoefer, Vienna Medical Academy, Vienna, 1971, pp. 155–162.
10. A. Foldes, P.L. Jeffrey, B.N. Preston, and L. Austin, *Biochem. J.*, **126**, 1209 (1972).
11. H. Matsui, T. Kato, C. Yamamoto, K. Fujita, and T. Nagatsu, *J. Neurochem.*, **37**, 289 (1981).
12. C. Refshauge, P.T. Kissinger, R. Dreiling, L. Blank, R. Freeman, and R.N. Adams, *Life Sci.*, **14**, 311 (1974).
13. L. Felice, J.D. Felice, and P.T. Kissinger, *J. Neurochem.*, **31**, 1461 (1978).
14. O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
15. E. Josefsson and L. Akerstrom, *J. Chromatogr.*, **174**, 465 (1979).
16. G. Sperk, I. Galhaup, E. Schlogl, H. Hortnagel, and O. Hornykiewicz, *J. Neurochem.*, **35**, 972 (1980).
17. Y. Hashizume, T. Yamaki, and H. Hidaka, *Br. J. Pharmacol.*, **59**, 157 (1977).
18. J. Poggioli, M. Solier, and M. Beauvallet, in: "Catecholamines and Stress." Ed. by E. Usdin, R. Kvetnansky, and I.J. Kopin, Pergamon Press, Oxford, 1976, pp. 389–393.
19. R.W. Fuller, H.D. Snoddy, and K.W. Perry, *Life Sci.*, **31**, 563 (1982).

Received 15 February 1988