Synthesis and Hypoglycemic Activity of Benzamidophenyl-alkanoic Acid Derivates: New Inhibitors of Gluconeogenesis

ERICH RAPP and HORST P. O. WOLF ˣ

Received July 21, 1981, from the *Research Laboratories of Byk Gulden Lomberg Pharmaceuticals, D-7750 Konstanz, Federal Republic of Germany.* Accepted for publication October 13, 1981.

Abstract: □ A series of ω-[2-(*N*-alkylbenzamido)-phenyl]-alkanoic acids was synthesized and tested for its effects on blood glucose concentration in fasted rats and on gluconeogenesis from lactate and pyruvate in isolated perfused rat livers. The compounds led to a dose-dependent and reversible inhibition of gluconeogenesis, with 4-[2-(*N*-methyl-3-trifluoromethylbenzamido)-phenyl]-butanoic acid leading to a 50% inhibition at 0.02 m*M*. The compounds lowered blood glucose in fasted rats. No correlation between hypoglycemic effect and inhibition of gluconeogenesis could be detected, however.

Keyphrases □ Benzamidophenyl-alkanoic acid—derivatives, synthesis, hypoglycemic activity □ Gluconeogenesis—inhibited by synthesized benzamidophenyl-alkanoic acid derivatives □ Hypoglycemic activity—benzamidophenyl-alkanoic acid derivatives, new inhibitors of gluconeogenesis

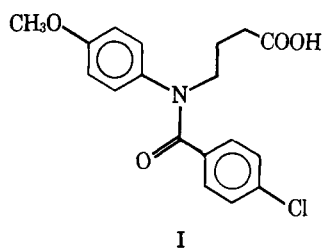
Numerous substances have been reported to lower blood glucose levels by inhibiting gluconeogenesis in mammalian liver; various sites of inhibition have been discussed (1). So far, only biguanides have been used as therapeutic agents in the treatment of diabetes.

Recently, clanobutin [4-[4-chloro-*N*-(4-methoxyphenyl)-benzamido]butanoic acid, (I)[1]] (2) was found to be an inhibitor of gluconeogenesis from the precursors lactate and pyruvate (3). This led to the synthesis of a series of structurally related compounds in order to find more potent inhibitors of gluconeogenesis as potential hypoglycemic drugs.

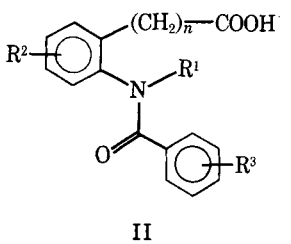
In variation of structure I, the alkanoic acid chain was attached to the aromatic ring *ortho* to the amide nitrogen, which was kept substituted with a methyl or ethyl group. Chain length and substitution pattern were modified (Table I).

EXPERIMENTAL

Chemistry—*3-[2-(N-Methyl-4-chlorobenzamido)-phenyl]propionic Acid (IIb)*—*N*-Methyldihydrocarbostyril (III*b*) 4.84 g (30 mmoles) and 5.6 g (100 mmoles) of potassium hydroxide were dissolved in 20 ml of ethylene glycol monoethyl ether. While stirring, the mixture was refluxed for 3 hr. After cooling to room temperature, the solution was diluted with 200 ml of water and extracted with two portions of ether. The pH of the aqueous phase was brought to 8–8.5 by addition of 2 *N* HCl. A solution of 5.25 g (30 mmoles) of 4-chlorobenzoylchloride (IV*b*) in 50 ml of ether was added slowly, while the pH of the aqueous phase was held at 7.5–8.5 with 1 *N* NaOH. The aqueous layer was separated and washed with ether twice. The resulting acid was precipitated with 2 *N* HCl, washed with water, and dried. Recrystallization from ethyl acetate yielded 6.2 g of II*b*, mp 156–158° (66%). Compounds II*d*, *e*, *g*, *h*, *k*, and *m–u* were synthesized by the same procedure.



I



II

4-[2-(N-Methylbenzamido)phenyl]butanoic Acid (IIh)—2-Aza-2-methyl-benzo[*c*]-cycloheptanone (III*h*) [14.0 g (80 mmoles)], 17.9 g (320 mmoles) of potassium hydroxide, and 50 ml of ethylene glycol monoethyl ether were brought together in a glass pressure vessel and heated to 150° for 6 hr. After cooling to room temperature, the contents were diluted with 200 ml of water and adjusted to pH 9 with glacial acetic acid. After stirring 33.6 g (400 mmoles) of sodium bicarbonate into this solution, 11.8 g (80 mmoles) of benzoyl chloride in 20 ml of ether was added dropwise within 2 hr. Dilution with 800 ml of water was followed by extraction with ether. The aqueous phase was acidified with 2 *N* HCl and extracted three times with ethylene chloride. The organic fractions were collected and concentrated; the residue was recrystallized from ethyl acetate–petroleum ether (1:1) to give 19.6 g of II*h*, mp 102–103° (80%). Compounds II*a*, *b*, *c*, *f*, *i*, *j*, and *l* were prepared according to this method.

6-[2-(N-Methyl-3-trifluoromethylbenzamido)-phenyl]caproic Acid (IIu)—Oxalyl chloride [13.0 g (102 mmoles)] and one drop of dimethyl formamide were given to 12.5 g (34 mmoles) of 4-[2-(*N*-methyl-3-trifluoromethylbenzamido)-phenyl]butanoic acid (III*l*) in 40 ml of toluene. The mixture was stirred for 1.5 hr and then evaporated *in vacuo*. The residue was taken up in 50 ml of dry dioxane and added dropwise to 1.32 g (35 mmoles) of sodium borohydride in 20 ml of dioxane. The mixture was heated to 100° for 4 hr, then cooled to room temperature. Acidifying with 2 *N* HCl and dilution with 20 ml of water was followed by extraction of the product with two portions of ether. Evaporation left an oily residue (10.9 g), which was dissolved in 20 ml of pyridine and 10 ml of toluene and added to a solution of 11.8 g (61.7 mmoles) of *p*-toluenesulfonyl chloride in 40 ml of toluene; stirring at 40° was continued for 20 hr. After filtration, the solution was first washed with 2 *N* HCl and saturated sodium chloride solution, then dried and concentrated *in vacuo*. The tosylate was obtained as a viscous oil, was dissolved in 40 ml of ethanol, and added dropwise to a solution of 2.6 g (14.3 mmoles) of sodium diethyl malonate in 30 ml of ethanol. Refluxing for 24 hr was followed by removing ethanol. The residue, after extractive workup, yielded 4.1 g of a light brown oil. This was treated with potassium hydroxide in methanol–toluene (1:1) at room temperature (stirring for 30 hr). Workup led to 2.0 g of the free substituted malonic acid, which was decarboxylated by heating to 160° for 2.5 hr. After recrystallization from ether, 0.8 g of II*u*, mp 103–104° (2%) was obtained.

Pharmacology—*Hypoglycemic Activity* —Groups of 6–12 male Sprague-Dawley rats[2] (160–200 g) were fasted overnight. Using an oral tube, the substances were administered as aqueous, neutral solutions (10 ml/kg of body weight), with control rats receiving the same volume of 0.9% NaCl. The dosage range was 0.36–1.67 mmoles/kg of body weight. Blood samples were obtained prior to and at 2, 4, and 6 hr after administration by puncturing the retroorbital plexus. Blood glucose was measured by standard enzymatic procedure[3]. The results were expressed as relative change in comparison to the control group on log-transformed intraindividual ratios of treatment *versus* pretreatment. An effective dose, ED_{50} (25%) ± *SD*, was calculated from dose–response relationships by linear or nonlinear regression techniques. An ED_{50} of 25% indicated that 50% of the group showed at least a 25% decrease of blood glucose compared with controls. Intergroup comparison of change due to treatment was performed by ANOVA and subsequent Scheffé-contrasts or pairwise Student-Welch tests (Table I).

[1] Byk Gulden Lomberg, Pharmaceuticals, D-7750 Konstanz, Federal Republic of Germany. Synthesis see (2).

[2] Ivanovas 50; Ivanovas, Kisslegg, West Germany.

[3] Hexokinase/glucose-6-phosphate dehydrogenase (HK/G6PDH method).

0022-3549/82/0700-0796$01.00/0

Table I—Structure of Title Compounds: Effect on Blood Glucose

	R^1	R^2	R^3	n	Yield, %	m.p.[b]	Molecular Formula	Decrease of Blood Glucose[c], %	Analysis			
IIa[a]	$-CH_3$	—H	—H	2	39	122–124	$C_{17}H_{17}NO_3$	12[d,f]	calc. C 72.07	H 6.05	N 4.94	
									found C 72.15	H 6.07	N 4.90	
IIb	$-CH_3$	—H	4-Cl	2	66	156–158	$C_{17}H_{16}ClNO_3$	11	calc. C 64.25	H 5.08	N 4.41	Cl 11.16
									found C 64.00	H 5.14	N 4.18	Cl 11.35
IIc	$-CH_3$	—H	3,4-Cl_2	2	31	149–150	$C_{17}H_{15}Cl_2NO_3$	10	calc. C 57.97	H 4.29	N 3.98	Cl 20.13
									found C 57.99	H 4.36	N 3.91	Cl 20.17
IId	$-CH_3$	5-Cl	3,4-Cl_2	2	47	159–161	$C_{17}H_{14}Cl_3NO_3$	15[d,e]	calc. C 52.81	H 3.65	N 3.62	Cl 27.51
									found C 53.01	H 3.74	N 3.58	Cl 27.61
IIe	$-CH_3$	5-Cl	3-CF_3	2	55	124–126	$C_{18}H_{15}ClF_3NO_3$	15[d]	calc. C 56.04	H 3.92	N 3.63	Cl 9.19
									found C 55.81	H 3.81	N 3.86	Cl 9.33
IIf	$-C_2H_5$	5-CH_3	3,4-Cl_2	2	22	141–143	$C_{19}H_{19}Cl_2NO_3$	13[d]	calc. C 60.00	H 5.04	N 3.68	Cl 18.65
									found C 60.08	H 5.14	N 3.79	Cl 18.64
IIg	$-CH_3$	—H	2-phenyl	3	30	154–155	$C_{24}H_{23}NO_3$	8	calc. C 77.19	H 6.21	N 3.75	
									found C 77.06	H 6.23	N 3.52	
IIh	$-CH_3$	—H	—H	3	80	102–103	$C_{18}H_{19}NO_3$	9[d]	calc. C 72.71	H 6.44	N 4.71	
									found C 72.85	H 6.44	N 4.61	
IIi	$-CH_3$	—H	4-Cl	3	51	123–124	$C_{18}H_{18}ClNO_3$	20[d,f]	calc. C 65.16	H 5.47	N 4.22	Cl 10.69
									found C 65.05	H 5.52	N 4.23	Cl 10.67
IIj	$-CH_3$	—H	3,4-Cl_2	3	61	120–121	$C_{18}H_{17}Cl_2NO_3$	19[d,f]	calc. C 59.03	H 4.68	N 3.82	Cl 19.36
									found C 59.09	H 4.58	N 3.81	Cl 19.58
IIk	$-CH_3$	—H	2,4-Cl_2	3	12	102–103	$C_{18}H_{17}Cl_2NO_3$	17[d,e]	calc. C 59.03	H 4.68	N 3.82	Cl 19.36
									found C 58.98	H 4.73	N 3.73	Cl 19.28
IIl	$-CH_3$	—H	3-CF_3	3	30	84–85	$C_{19}H_{18}F_3NO_3$	20[d,f]	calc. C 62.46	H 4.97	N 3.83	F 15.60
									found C 62.36	H 4.92	N 3.89	F 15.40
IIm	$-CH_3$	—H	2,6-Cl_2	3	20	161–163	$C_{18}H_{17}Cl_2NO_3$	3	calc. C 59.03	H 4.68	N 3.82	Cl 19.36
									found C 58.90	H 4.73	N 4.06	Cl 19.38
IIn	$-CH_3$	6-OCH_3	4-Cl	3	66	121–122	$C_{19}H_{20}ClNO_4$	14[d,e]	calc. C 63.07	H 5.57	N 3.87	Cl 9.80
									found C 62.84	H 5.65	N 3.84	Cl 9.81
IIo	$-CH_3$	4-OCH_3	4-Cl	3	31	107–108	$C_{19}H_{20}ClNO_4$	15[d,e]	calc. C 63.07	H 5.57	N 3.87	Cl 9.80
									found C 63.07	H 5.44	N 3.79	Cl 9.63
IIp	$-CH_3$	4-OCH_3	2,4-Cl_2	3	38	90–91	$C_{19}H_{19}Cl_2NO_4$	12[d,e]	calc. C 57.59	H 4.83	N 3.54	Cl 17.89
									found C 57.77	H 4.91	N 3.44	Cl 17.98
IIq	$-CH_3$	5-OCH_3	3,4-Cl_2	3	39	87–88	$C_{19}H_{19}Cl_2NO_4$	21[d,f]	calc. C 57.59	H 4.83	N 3.54	Cl 17.89
									found C 57.41	H 4.98	N 3.51	Cl 17.61
IIr	$-CH_3$	—H	4-Cl	4	44	117–118	$C_{19}H_{20}ClNO_3$	9[d]	calc. C 65.99	H 5.83	N 4.05	Cl 10.25
									found C 65.85	H 5.73	N 4.01	Cl 10.34
IIs	$-CH_3$	—H	3-CF_3	4	50	81–83	$C_{20}H_{20}F_3NO_3$	21[d,f]	calc. C 63.32	H 5.31	N 3.69	
									found C 63.44	H 5.06	N 3.84	
IIt	$-CH_3$	—H	3,4-Cl_2	4	30	86–87	$C_{19}H_{19}Cl_2NO_3$	5	calc. C 60.01	H 5.04	N 3.68	Cl 18.65
									found C 60.21	H 5.16	N 3.74	Cl 18.26
IIu	$-CH_3$	—H	3-CF_3	5	2	103–104	$C_{21}H_{22}F_3NO_3$	4	calc. C 64.11	H 5.64	N 3.56	
									found C 64.24	H 5.90	N 3.58	

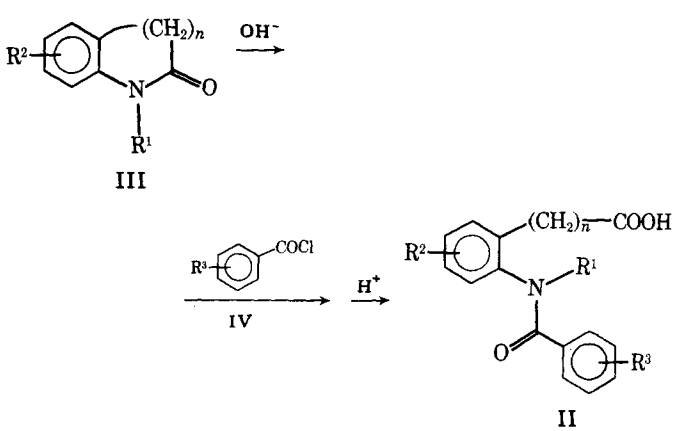
[a] All reported compounds show NMR spectra in accordance with expected structures. [b] Melting points are not corrected. [c] Percent decrease of blood glucose compared with controls after oral administration of 0.06 mmole/kg; the values represent the maximum decrease within 6 hr after administration, 6 rats/group. [d] Statistical tests: ANOVA and subsequent Scheffé-contrasts or pairwise Student-Welch tests with $p < 0.05$, two-sided. [e] $p < 0.01$, two-sided. [f] $p < 0.001$, two-sided.

Liver Perfusion—The isolated livers of rats fasted overnight were perfused in a nonrecirculating system (4). Krebs-Henseleit bicarbonate buffer (pH 7.4), saturated with an oxygen–carbon dioxide mixture (95:5) and containing L(+)-lactate[4] (1.6 m*M*) and pyruvate[5] (0.2 m*M*), was used for perfusion. The livers were perfused for 2 hr; the test substances were infused from 32–80 min using three different, increasing concentrations at 16-min intervals.

Analytical Method and Determination of K_i—Samples of the liver perfusion effluent were collected at 1-min intervals and analyzed for glucose. For each experiment, the values observed at 32, 48, 64, 80, and 96 min were obtained by averaging five neighboring values. The slight linear decline found in control experiments was taken into account by using the values at 32 and 96 min as a basis for linear interpolation. The concentration producing a 50% decrease in glucose due to inhibition of gluconeogenesis (K_i) was calculated from these data by linear or log–linear correlation.

Acute Toxicity—Five female mice[6] (22–26 g) were used for every group. The animals received water *ad libitum;* food was reduced to 50 g/kg of body weight 18 hr prior to administration. The test compounds were administered orally. LD_{50} values were calculated according to a previous study (5).

Synthesis—The title compounds were synthesized as follows: According to the known procedures, benzolactams (III) with varying ring size were prepared from substituted anilines (*via* internal Friedel-Crafts alkylation) (6), tetralones, and benzosuberones (*via* Beckmann or Schmidt rearrangement) (7, 8); the resulting lactams were *N*-alkylated to III with dimethyl- or diethylsulfate either directly or by use of phase-transfer catalysis (9). Benzolactams (III) were then hydrolyzed by refluxing with potassium hydroxide in ethylene glycol monoethyl ether; in some cases, glass pressure vessels were used for even higher reaction temperatures. Acylation of the amino group with substituted benzoyl chlorides (IV) and acidic workup led to compounds IIa–*t*. One



Scheme I

[4] K. Roth OHG, Karlsruhe, West Germany.
[5] Boehringer GmbH, Mannheim, West Germany.
[6] NMRI strain.

Table II—Biological Data and Toxicity

	Blood Glucose Decrease ED_{50} (25%) ± *SD* (*n*),[a] mmole/kg	Inhibition of Gluconeogenesis in Isolated Perfused Rat Liver, K_i ± *SD*, m*M*	Toxicity LD_{50} (oral) Mice, mmole/kg
II*a*	1.38 ± 0.13 (180)	0.68 ± 0.15	5.3
II*b*	>1.57 (29)	0.18	—
II*c*	>1.70 (60)	0.06	3.1
II*d*	not feasible (30)	0.03	—
II*e*	not feasible (60)	0.11	>2.6
II*f*	—	0.08	—
II*h*	1.51 ± 0.15 (180)	0.15 ± 0.05	>5.1
II*i*	0.99 ± 0.18 (180)	0.18 ± 0.03	3.9
II*j*	1.26 ± 0.17 (120)	0.05 ± 0.01	2.1
II*k*	1.66 ± 0.01 (60)	0.06	>2.7
II*l*	not feasible (60)	0.02	—
II*n*	not feasible	0.04	—
II*q*	not feasible	0.08	—
II*t*	not feasible	0.05	—
Buformin[a]	1.00 ± 0.40 (60)	>1.00	2.46

[a] *N* = number of animals tested. [b] 1-butylbiguanide hydrochloride.

further example, II*u*, was prepared by extension of the alkyl chain *via* malonic ester synthesis (Scheme I).

RESULTS AND DISCUSSION

Compounds II*a–u* were tested for their ability to lower blood glucose levels in fasted rats and to inhibit gluconeogenesis from lactate and pyruvate in the model isolated perfused liver of fasted rats.

The decrease of blood glucose after a single oral dose of 0.6 mmole/kg in overnight fasted rats was determined. Table I shows the maximum value within 6 hr after administration.

For a number of compounds, dose–response relationships were studied by calculating ED_{50} (25%) values from the corresponding data (dosage range 0.36–1.67 mmoles/kg). A dose–response relationship could not be found in all cases, *i.e.*, the ED_{50} value was not feasible (Table II).

The inhibition of gluconeogenesis from L(+)-lactate (1.6 m*M*) and pyruvate (0.2 m*M*) in the liver perfusion experiment is expressed by the inhibition constant K_i, *i.e.*, the concentration producing a 50% inhibition (Table II).

Acute toxicity data were determined for selected compounds (Table II).

The *in vivo* glucose lowering effects of the reported compounds were found to be in the same order of magnitude as the 1-butylbiguanide hydrochloride[7]. The butanoic acid derivatives (II*i* and *j*) showed the lowest ED_{50} (25%) values: 0.99 and 1.26 mmoles/kg.

Effective inhibitions of gluconeogenesis, however, were demonstrated by most members of the group, the best of which are II*d*, *j*, *l*, *n*, and *t* ($K_i \leq 0.05$ m*M*). Inhibition was fully reversible without delay upon terminating the infusion of the inhibitor substance. This coincides with results obtained on I (3). In comparison, 1-butylbiguanide hydrochloride fails to show a substantial effect upon gluconeogenesis in this model up to 1.0 m*M*.

Neither structure–activity relationships within the group II*a–u* nor any correlation between hypoglycemic activity and K_i values could be found.

Unsubstituted ω-phenylalkanoic acids have been reported to inhibit glucose synthesis from various precursors (10). 4-Phenylbutanoic acid effects a 60% inhibition at 4 m*M*. Structurally closely related to II are some 2-benzamidophenylacetic and -propionic acids, which were found to possess anti-inflammatory and analgesic activity (11).

Other inhibitors reported within the last 10 years include cyclopropanecarboxylic acid (12), phenelzine (13), tryptophan metabolites (14), 3-mercaptopicolinic acid (15), and aminopyrine (4), all effective in the range of 0.1–0.5 m*M*; 0.6 m*M* is reported for 2-bromooctanoate (16), 0.5–1.0 m*M* for pent-4-enoic acid (17), 2 m*M* for phenylpyruvate (18), and 5 m*M* for butylmalonate (19).

CONCLUSION

The data presented in Tables I and II indicate that some of the substituted ω-(2-benzamidophenyl)alkanoic acids of type II lower the blood glucose level *in vivo* significantly, while a larger group of compounds inhibit gluconeogenesis *in vitro*. Although no obvious correlation seems to exist between the two groups, a few substituted butanoic acids are effective in both models, especially II*j*, which, in addition to interesting ED_{50} and K_i values, possesses the lowest toxicity of the compounds measured. The inhibition constants of II*d*, *l*, and *n*, relatively nontoxic substances, belong to the lowest K_i values reported.

REFERENCES

(1) K. Klemm, W. Krastinat, and U. Krüger, *Drug Res.*, **29(I)** 1 (1979).

(2) M. S. A. Sherratt and L. Hue, *Biochem. Soc. Trans.*, **7**, 99 (1979).

(3) H. P. O. Wolf, J. Schlepper, V. Steinijans, and R. Scholz, *Biochem. Pharmacol.*, **29**, 1649 (1980).

(4) R. Scholz, W. Hansen, and R. G. Thurman, *Eur. J. Biochem.*, **38**, 64 (1973).

(5) J. T. Lichtfield, Jr. and F. W. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

(6) F. Mayer, L. van Zütphen, and H. Philipps, *Chem. Ber.*, **60**, 858 (1927).

(7) F. Möller in "Methoden der Organ. Chemie," E. Müller, Ed., Thieme-Verlag, Stuttgart, 1957, vol. XI, part 1, p. 892.

(8) M. Tomita, S. Minami, and S. Uyeo, *J. Chem. Soc. C*, **1969**, 183.

(9) R. Brehme, *Synthesis*, **1976**, 113.

(10) L. G. DeGaldeano, R. Bressler, and K. Brendel, *J. Biol. Chem.*, **248**, 2514 (1973).

(11) D. J. Drain, M. J. Daly, B. Davy, M. Horlington, J. G. B. Howes, J. M. Scruton, and R. A. Selway, *J. Pharm. Pharmacol.*, **22**, 684 (1970).

(12) J. J. Bahl, K. Brendel, and R. Bressler, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **38**, 113, 149 (1979).

(13) R. Haeckel and M. Oellerich, *Eur. J. Clin. Invest.*, **7**, 393 (1977).

(14) S. A. Smith, K. F. R. Elliott, and C. Pogson, *Biochem. Pharmacol.*, **28**, 2145 (1979).

(15) M. N. Goodman, *Biochem. J.*, **150**, 137 (1975).

(16) B. M. Raaka and J. M. Lowenstein, *J. Biol. Chem.*, **254**, 3303 (1979).

(17) N. B. Rudermann, C. J. Toews, C. Lowy, I. Vreeland, and E. Shafrir, *Am. J. Physiol.*, **219**, 51 (1970).

(18) I. J. Arinze and M. S. Patel, *Biochemistry*, **12**, 4473 (1973).

(19) J. R. Williamson, J. Anderson, and E. T. Browning, *J. Biol. Chem.*, **245**, 1717 (1970).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the expert technical assistance of F. Deppermann, M. Eisele, P. Lamm, and H. Merkel; and thank Dr. H. Müller for performing the toxicity studies.

[7] Batch no. 2036, Società Italiana Medicinali Scandicci, Italy.