(6) A. B. Segelman, *J. Chromatogr.*, **82**, 151(1973).
(7) R. D. Sofia, R. K. Kubena, and H. Barry, III, *J. Pharm. Pharmacol.*, **23**, 889(1971).
(8) N. B. Eddy and D. Leimbach, *J. Pharmacol. Exp. Ther.*, **107**, 385(1953).
(9) J. T. Litchfield, Jr., and F. Wilcoxon, *ibid.*, **96**, 99(1949).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 17, 1974, from the *Department of Pharmacognosy, College of Pharmacy, Rutgers—The State University, New Brunswick, NJ 08903, and the ‡Department of Pharmacology, Wallace Laboratories, Cranbury, NJ 08512

Accepted for publication February 26, 1974.

Supported by Research Grant RO1-DA-00328 from the National Institute on Drug Abuse, U.S. Public Health Service.

The authors are grateful to Dr. Monique C. Braude, Executive Secretary, NIMH-FDA Psychotomimetic Agents Advisory Committee, for arranging procurement of marijuana and authentic cannabinoid substances.

x To whom inquiries should be directed.

Effect of γ-Radiation on Yield of Insulin from Beef Pancreas Glands

PAUL SKIERKOWSKI*x and MARTIN BEASLEY‡§

Abstract □ Beef pancreas glands were subjected to varying doses of γ-radiation to inactivate proteolytic enzymes and thus allow greater insulin yields *via* normal extraction procedures. An increase in yield was seen with doses greater than 10,000 rads, with the maximum yield obtained from a 20,000-rad dose.

Keyphrases □ Radiation, gamma—effect on yield of insulin from beef pancreas glands □ Insulin—increased yields by γ-irradiation of beef pancreas glands

Recent beef shortages as well as recommended restrictions by the Food and Drug Administration on the use of oral hypoglycemics (1) have placed a serious strain on the supply of insulin from cattle and hogs. Although an increase in animal slaughter will somewhat alleviate this shortage, an increasing population carries with it an increasing demand for insulin. Thus, there is a need for methods of increasing the yield of insulin extracted from the pancreas glands of these animals.

The commercial insulin procedure is a combination of acid-alcohol extractions and isoelectric precipitations to achieve a purified insulin (2). Frozen glands in 45.9–68.1-kg (100–150-lb) lots are ground and placed in large extraction tanks filled with cold acidified alcohol. The acid–alcohol extracts the insulin from the glands and inactivates enzymes that could destroy the insulin in the extract. This step is most important, and it is felt that a loss of insulin in the grinding–extraction process could still be due to some action of the proteolytic enzymes on the insulin. Thus, if enzymes could be further inactivated, less degradation of insulin would occur and the yield would be improved.

One such means of inactivating the enzymes would be through the use of ionizing radiation (3). Irradiation of frozen glands prior to the grinding step could inactivate the enzymes that may be degrading the insulin during the subsequent steps.

Table I—Results of Insulin Assays of Irradiated Pancreas Glands

Sample Dose, rads	Yield, Units/ 0.454 kg	Change from Control, %	*p*
0 (Control)	1456 ± 54	—	—
4,000	1469 ± 45	+0.9	—
6,000	1393 ± 48	−4.3	<0.02
8,000	1278 ± 55	−12.2	<0.001
10,000	1457 ± 102	0	—
12,000	1742 ± 104	+19.6	<0.001
14,000	1702 ± 123	+16.9	<0.001
16,000	1657 ± 126	+13.8	<0.001
18,000	1831 ± 155	+25.8	<0.001
20,000	1954 ± 160	+34.2	<0.001
50,000	1820 ± 80	+25	<0.001
100,000	1490 ± 174	+2.03	—

EXPERIMENTAL

Approximately 11.6 kg (25 lb) of beef pancreas glands was obtained from a commercial slaughterhouse. The glands had been collected during the previous day's slaughter and were stored at 5°. These glands were transported in a refrigerated container to the laboratory and then placed in a −10° freezer for 24 hr. The frozen glands were chopped into pieces approximately 2.54–5.08 cm (1–2 in.) square, and all pieces were uniformly mixed. This procedure was done to approximate a uniform sample and avoid individual gland variation. Lots (0.2 kg, 0.5 lb) of the mixed frozen glands were then placed in polyethylene bags for irradiation.

Samples (0.9 kg, 2 lb) of the glands were then irradiated in 0.2-kg (0.5-lb) lots in a cobalt-60 (^{60}Co) pool-type irradiator[1] (7 cm i.d.; 1.85×10^2 rads/sec) for varying doses. All samples remained frozen during the irradiation. There was no induced radioactivity in the sample as determined by using a NaI (Tl) detector. Following irradiation, the glands were ground, weighed, and extracted with cold (1–4°) 95% ethanol acidified to pH 2.5 with concentrated phosphoric acid. The extraction time was 30 min and the temperature of the final extraction mixture was measured; then this mixture was filtered to collect the crude alcoholic extract. Ten aliquots of the alcoholic filtrate were collected for assay.

The radioimmunoassay technique of Herbert *et al.* (4) was uti-

[1] Lockhead-Georgia Co., Marietta, Ga.

lized to determine the insulin activity of the filtered extracts. A standard curve was prepared with dilutions of USP insulin reference standard. Triplicate samples of the extraction aliquots were then assayed, and data were converted to original units of insulin per unit weight of pancreas glands.

RESULTS AND DISCUSSION

Irradiation of the beef pancreas glands at doses between 10,000 and 20,000 rads produced significant increases in insulin yield when compared to the control sample (Table I). Beginning at 12,000 rads, an increase in yield occurs. The maximum yield obtained was an increase of 34.2% over the control with a dose of 20,000 rads.

From the data in Table I, it appears that 20,000 rads is the most effective dose of ^{60}Co γ-radiation for increasing insulin yields. Although it remains to be proven that it is a specific enzyme inactivation that results in the increased yield, this effect of γ-irradiation can be hypothesized. It is also possible that the irradiation could affect the insulin molecule. However, experiments conducted in this laboratory using reference standards in simulated gland concentrations have shown no significant loss in insulin as a result of γ-irradiation in these dose ranges. The insulin molecule, a small protein of molecular weight of approximately 6000, is much smaller than most enzymes. In addition, enzymes usually have unique subgroups attached to them which make them more susceptible to the effects of ionizing radiation (4).

The radioimmunoassay method used in this experiment does not assure the safety of these insulin extracts in humans. Further biological testing is necessary to allow clinical application. However, considering the present factors of increased population and increased utilization of insulin, in lieu of oral hypoglycemic agents with reported side effects, utilization of γ-radiation to increase the insulin yield of pancreas glands may be a means of increasing this insulin supply.

REFERENCES

(1) "FDA Drug Bulletin," U.S. Department of Health, Education, and Welfare, May 1972.

(2) "Diabetes Mellitus," 7th ed., Eli Lilly and Co., Indianapolis, Ind., 1970.

(3) D. S. Grosch, "Biological Effects of Radiations," Blaisdell, New York, N.Y., 1965.

(4) V. Herbert, K. S. Law, C. W. Gottlieb, and S. J. Bleicher, *J. Clin. Endocrinol.*, **25**, 1375(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 28, 1973, from the * *School of Pharmacy, University of Mississippi, University, MS 38677,* and the ‡ *School of Pharmacy, Auburn University, Auburn, Ala.*

Accepted for publication March 8, 1974.

Supported in part by the National Science Foundation Undergraduate Research Program, the Research Institute of Pharmaceutical Sciences, and the Committee on Faculty Research, University of Mississippi.

The authors thank Mrs. Aurora C. McDonnough for technical assistance.

§ National Science Foundation Undergraduate Research Program Participant, 1973, University of Mississippi.

ˣ To whom inquiries should be directed.

New Compounds: Monoesters of α-Aminobenzylphosphonic Acid

W. FRANKLIN GILMOREˣ and HILMER A. McBRIDE

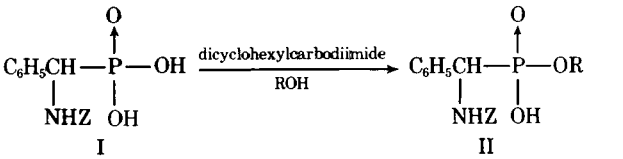
Abstract □ The reaction of α-(*N*-carbobenzoxyamino)benzylphosphonic acid with *n*-propanol or ethanol in the presence of triethylamine and dicyclohexylcarbodiimide gave the title compounds in good yields.

Keyphrases □ α-Aminobenzylphosphonic acid monoesters—synthesis □ Phosphonic acid monoesters, α-aminobenzyl—synthesis

While investigating methods of incorporating α-aminophosphonic acids into peptides, the conversion of aminophosphonic acids to monoesters became of interest. It is well known that phosphonic acids can be converted to monochlorides by use of 1 equivalent of phosphorus pentachloride. Addition of the monochlorides to alcohols furnishes the monoesters (1). While this method is satisfactory, it is laborious. Monoalkyl esters of *N*-substituted α-aminophosphonic acids may be prepared by treating a Schiff base with an alkaline salt of an alkyl dihydrogen phosphonate (2). This method is not satisfactory for preparing the monoesters of α-(*N*-carbobenzoxyamino)phosphonic acids. Partial hydrolysis of the diesters of aminophosphonic acids has been used as a source of the monoesters (3).

Dicyclohexylcarbodiimide has been used for the synthesis of esters of phosphorus acids (4–8). The preparation of monoesters from α-(*N*-carbobenzoxyamino)benzylphosphonic acid (I) by treatment with alcohols and dicyclohexylcarbodiimide is the subject of this report. Scheme I illustrates this synthesis.

The α-(*N*-carbobenzoxyamino)benzylphosphonates (II) can be converted to the deblocked amine hydrobromides by treatment with hydrogen bromide in acetic acid. The water-soluble hydrobromide salts can be converted to the amino esters by use of silver oxide.



I

Z = carbobenzoxy

II

R = ethyl or propyl

Scheme I