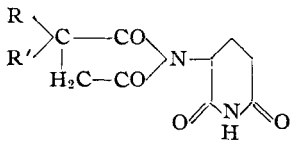
Derivatives of Glutarimide Likely to Possess Therapeutic Activity

By S. EL-ZANFALLY, M. KHALIFA, and Y. M. ABOU-ZEID

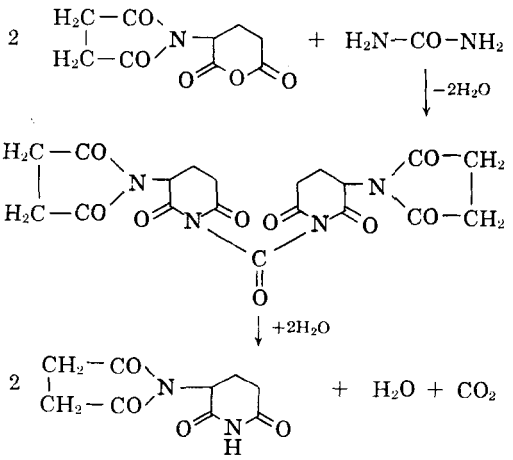
The synthesis of certain α-substituted glutarimide derivatives is described. A mechanism is suggested for the imidation of the inner anhydrides with urea.

ALTHOUGH glutarimide itself is therapeutically inactive, α-substituted derivatives *e.g.*, α-ethyl-α-phenyl glutarimide is therapeutically active and used in medicine as a sedative and hypnotic (1). Meanwhile derivatives of succinimide such as α-ethyl-α-methyl succinimide and α-phenyl-*N*-methyl succinimide are used as anticonvulsants and in petit mal (2, 3). Furthermore, combining hypnotics and anticonvulsants is a common practice in the treatment of grand mal. Consequently the idea of synthesizing compounds with glutar- and succinimido moieties (I) emerged and seemed most attractive.



R and R′ may be the same or different.
R and R′ = H, CH_3, C_6H_5.

Derivatives having the above general formula were synthesized by condensing succinic anhydride or the α-substituted derivatives with glutamic acid. The product thus obtained was converted to the anhydride by heating with acetic anhydride and then to the corresponding imide either by fusion with urea under pressure or by the ammonium carbonate method. α,α-Dimethylsuccinic acid was prepared in 70% yield according to a method (4) described in the literature, and it was converted to the anhydride in 80% yield by the acetyl chloride method (5). The α-phenyl acid was prepared by Higson and Thorpe's procedure (6) in an over-all yield of 78%, calculated on the mandilonitrile. An attempt to convert it to the anhydride by the method of Dehn and Thorpe (7) did not give satisfactory results. However, the anhydride was obtained in 80% yield by heating the acid with excess acetic anhydride at 100° for 8 hr. instead of heating to 140° for 6 hr. as recommended by Thorpe. Billman and Harting in their work on the acylation of glutamic acid with inner anhydrides (8) reported that they were able to prepare the *N*-phthalyl derivative by fusing phthalic anhydride with glutamic acid, whereas the *N*-succinoyl derivative could not be obtained by the same procedure. However, in the present investigation, succinoylation of glutamic acid was achieved by refluxing glutamic acid with succinic anhydride or the α-substituted derivatives in dry pyridine. Attempts to isolate the acylated glutamic acid derivatives were unsuccessful. Accordingly they were converted directly to the anhydride by heating with acetic anhydride. Several attempts were made to convert the anhydrides to the corresponding imides. Leonard's procedure (9), which consists of heating the anhydride gradually with 70% aqueous ethylamine, proved to be unsatisfactory, while the urea–dry xylene method (10) was unsuccessful. Nevertheless, the imides, except α-(phenylsuccinimido)glutarimide, were obtained by fusing the anhydrides with urea under pressure. The method is essentially that described in a German patent (11). The formation of the imide probably takes place according to Scheme I.



Scheme I

Scheme I, besides being in accord with the fact that one molecule of urea is taken for each two molecules of the anhydride, gives a reasonable answer to the evolution of carbon dioxide in the course of the fusion.

α-(Phenylsuccinimido)glutarimide was prepared from the corresponding anhydride by the ammonium carbonate method (12), which proved to be superior to the urea method, since through its application the other two imides were obtained in much better yields.

EXPERIMENTAL[1]

α-Phenylsuccinic Anhydride.—α-Phenylsuccinic acid (20 Gm.) and redistilled acetic anhydride (60 ml.) were heated at 100° for 8 hr. Then the acetic acid and excess acetic anhydride were distilled at ordinary pressure and the residue was distilled *in vacuo*. The anhydride distilled over at 196°/16 mm., as a colorless oil which solidified on standing, m.p. 53–54° as reported (7); yield, 80%.

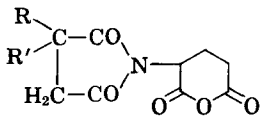
α-Succinimidoglutaric Anhydride and Derivatives.—These were prepared by the following general method.

A mixture of equimolecular amounts of succinic anhydride (or the α-substituted derivatives) and glutamic acid was suspended in dry pyridine (about 2 ml. pyridine per 1 Gm. of mixture) and refluxed to a clear solution after 1.5 hr. The pyridine was distilled off *in vacuo*, and the residue was boiled with double its weight of acetic anhydride for about 5 min. The anhydride, which separated out on cooling, was recrystallized from ethyl acetate. (See Table I.)

Received September 8, 1964, from the Faculty of Pharmacy, Cairo University, Cairo, Egypt.
Accepted for publication November 5, 1964.

[1] Melting points were performed by the capillary tube method and were uncorrected.

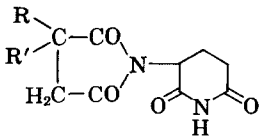
TABLE I.—α-SUCCINIMIDOGLUTARIC ANHYDRIDE AND DERIVATIVES



Anhydride	R	R′	Yield, %	m.p., °C	Formula	Anal.[b] C, % Calcd.	C, % Found	H, % Calcd.	H, % Found	N, % Calcd.	N, % Found
α-Succinimidoglutaric anhydride[a]	H	H	60	170	$C_9H_9NO_5$	51.18	51.05	4.27	4.40	6.63	6.70
α-(3,3-Dimethylsuccinimido)glutaric anhydride	CH_3	CH_3	80	176	$C_{11}H_{13}NO_5$	55.22	54.95	5.43	5.67	5.81	5.77
α-(Phenylsuccinimido)glutaric anhydride	H	C_6H_5	50	166	$C_{15}H_{13}NO_5$	62.72	62.67	4.53	4.61	4.88	4.91

[a] This anhydride was hydrolyzed to the corresponding acid in 80% yield by boiling with water, m.p. 156–157°. *Anal.*—Calcd. for $C_9H_{11}NO_6$: C, 47.20; H, 4.80; N, 6.11. Found: C, 47.39; H, 4.99, N, 6.07. [b] Analyses performed by Alfred Bernhardt, Germany.

TABLE II.—α-SUCCINIMIDOGLUTARIMIDE AND DERIVATIVES



Imide	R	R′	Method of Prepn.	Yield, %	Solvent of Crystallization[a]	m.p., °C	Formula	Anal.[b] C, % Calcd.	C, % Found	H, % Calcd.	H, % Found	N, % Calcd.	N, % Found
α-Succinimido glutarimide	H	H	*a*	20	A	221–222	$C_9H_{10}N_2O_4$	51.46	51.60	4.76	4.69	13.33	13.23
			b	80									
α-(3,3-Dimethylsuccinimido)-glutarimide	CH_3	CH_3	*a*	50	A	222–223	$C_{11}H_{14}N_2O_4$	55.42	55.55	5.88	6.05	11.76	11.82
			b	90									
α-(Phenylsuccinimido)-glutarimide	H	C_6H_5	*b*	50	B	188–190	$C_{15}H_{14}N_2O_4$	62.94	62.76	4.89	4.99	9.79	9.91

[a] A, aqueous alcohol; B, absolute alcohol. [b] Analyses performed by Alfred Bernhardt, Germany.

α-Succinimidoglutarimide and Derivatives.—These were obtained from the corresponding anhydrides by imidation either with urea or with ammonium carbonate according to the following general directions.

(*a*) *Urea Method.*—A mixture of the anhydride (2 moles) and urea (1 mole) was heated in a pressure bottle to 170–180° in an oil bath for 15 min. during which the fused mixture was shaken from time to time. The cooled, hard glossy mass was dissolved in boiling 50% aqueous ethanol. On cooling, the imide separated in a nearly pure state. Recrystallization from ethanol–water mixture afforded the pure imide.

(*b*) *Ammonium Carbonate Method.*—The anhydride (3 moles) and ammonium carbonate (4 moles) were mixed and heated gently to a state of quiet fusion in a flask fitted with an air condenser. The heating and occasional shaking were continued until effervescence ceased and a homogeneous melt was obtained (total heating time about 1 hr.). After being cooled, the hard glossy mass was dissolved in boiling alcohol from which the imide separated on cooling. (See Table II.)

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Stress Effects on Hyaluronidase Activity

By MICHAEL M. CLAY and CLAIRE R. SINAI

Rats maintained in a cold environment for 2 to 16 days showed decreased dermal hyaluronidase spreading activity, which was not accompanied by adrenal hypertrophy. Stress-free conditions for 24 hr. after cold caused a return toward prestress hyaluronidase spreading activity in rats treated with cold for 2, 4, and 8 days but not 16 days. Swimming for 1 hr. caused an increase in spreading activity in rats treated for 4, 8, and 16 days but a decrease at 2 days. These changes were followed by adrenal hypertrophy 24 hr. later. The results obtained with cold and cold followed by forced swimming are in agreement with those reported elsewhere with heat and heat followed by forced swimming, respectively.

SEVERAL STUDIES have shown that glucocorticoid hormones and stress decrease diffusibility of colloidal particles through connective tissue (1–3). This effect has been attributed to a decreased concentration or alteration in connective tissue hyaluronic acid (4).

Enhanced spread of a hemoglobin–hyaluronidase solution has been reported by Hayes and Baker (5) in the skins of rats treated for 37 days with glucocorticoid hormones. To account for the increased spread, the authors suggested that the substrate, hyaluronic acid, was altered structurally or reduced in amount. However, Clay and Nelson (6) reported that rats stressed with heat for as long as 4 months failed to demonstrate increased hyaluronidase spreading activity. Clay and Nelson reported further that a single injection of cortisone acetate (7) or forced swimming for 2.25 hr. (8) increased hyaluronidase spreading activity in rats previously stressed with heat for 7 weeks.

The following study was undertaken to determine whether prolonged cold treatment followed by forced swimming would duplicate the effects of heat and swimming described above.

METHODS

Seventy-two male Wistar rats were bred in our animal quarters. They were assigned randomly to nine equal groups.

Received May 14, 1964, from the Division of Pharmacology and Animal Sciences, College of Pharmacy, Columbia University, New York, N. Y.
Accepted for publication November 17, 1964.
The authors thank G. D. Searle and Co. for the supply of hyaluronidase, trademarked Alidase.

Dermal diffusibility was assayed in a control group by injecting a hyaluronidase–India ink solution into the skin. These rats were sacrificed 24 hr. later. Four groups of rats were maintained in a cold chamber for 2, 4, 8, or 16 days. Dermal diffusibility of hyaluronidase–India ink was assayed in each rat 24 hr. before and 2 hr. after cold treatment. They were sacrificed 24 hr. after removal from the cold. The remaining four groups were treated with cold for 2, 4, 8, or 16 days, and dermal diffusibility was assayed 24 hr. before and 2 hr. after treatment. However, 24 hr. after removal from the cold, dermal diffusibility was assayed again. Two hours later, the rats were forced to swim for 1 hr. in water maintained at 25 ± 1°. Two hours later, a fourth assay of diffusibility was made. The rats were sacrificed 22 hr. later.

Dermal diffusibility was assayed by the method of Clay and Nelson (6). A volume of 0.05 ml. of an indicator solution was injected into the skin of the flank. The solution contained 150 U.S.P. units of hyaluronidase, 17% India ink, and 0.9% sodium chloride in distilled water. The area of spread was calculated by substituting the longest and widest dimensions of the ink spot in the formula for an ellipse. Measurements were made 22 or 24 hr. after injection under light ether anesthesia.

During cold treatment, the rats were maintained in an environment at 8 ± 2° in individual wire mesh cages (10 in. in diameter, 5 in. high) in a dark ventilated chamber. Free access to food and water was provided.

At all times other than during cold treatment the