TABLE I

TRISUBSTITUTED CARBINYLCARBAMATES OF FORMULA RR'R''C–NHCOO$(CH_2)_nR'''$

R	R'	R''	R'''	n	B.p., °C.	Mm.	n_D	t, °C.	Yield, %	Formula	Nitrogen, % Calcd.	Found
C_2H_5	C_2H_5	C_2H_5	$N(C_2H_5)_2$	2	128–129	1	1.4560	25	62	$C_{14}H_{30}O_2N_2$	10.85	10.85
C_2H_5	C_4H_9	C_4H_9	$N(C_2H_5)_2$	2	164–166	2.5	1.4568	25	83	$C_{18}H_{38}O_2N_2$	8.91	8.87
C_4H_9	C_4H_9	C_4H_9	$N(CH_3)_2$	2	175–176	5	1.4570	22	83	$C_{18}H_{38}O_2N_2$	8.91	9.31
C_4H_9	C_4H_9	C_4H_9	$N(C_2H_5)_2$	2	164–165	2	1.4567	25	87	$C_{20}H_{42}O_2N_2$	8.18	8.31
C_4H_9	C_4H_9	C_4H_9	$N(C_4H_9)_2$	2	181–183	2	1.4550	22	83	$C_{24}H_{50}O_2N_2$	7.03	7.17
C_4H_9	C_4H_9	C_4H_9	$N(C_4H_9)_2$	3	185–190	3	1.4531	24	80	$C_{25}H_{52}O_2N_2$	6.79	6.78
C_4H_9	C_4H_9	C_4H_9	$N(C_2H_5)_2$	3	175–177	2	1.4558	22	75	$C_{21}H_{44}O_2N_2$	7.86	7.78
C_4H_9	C_4H_9	C_4H_9	$CH_2CH(CH_3)N(C_2H_5)_2$	0	164–165	1.5	1.4548	23	46	$C_{21}H_{44}O_2N_2$	7.86	8.06
C_4H_9	C_4H_9	C_4H_9	$CH_2CH_2NC_5H_{10}$[a]	0	185–188	3	1.4682	25	64	$C_{21}H_{42}O_2N_2$	7.90	8.04
C_4H_9	C_4H_9	C_4H_9	$CH_2CH_2C_5H_4N$[b]	0	198–200	2	1.4877	24	61	$C_{21}H_{36}O_2N_2$	8.04	8.05
C_5H_{11}	C_5H_{11}	C_5H_{11}	$N(C_2H_5)_2$	2	180–183	2.5	1.4564	25	82	$C_{23}H_{48}O_2N_2$	7.29	7.17
$(C_2H_5)_2NCH_2$	C_4H_9	C_4H_9	$N(C_2H_5)_2$	2	186–187	4.5	1.4603	25	85	$C_{21}H_{45}O_2N_3$	11.32	11.10
$C_5H_{10}NCH_2$[c]	C_4H_9	C_4H_9	$N(C_2H_5)_2$	2	186–188	4	1.4720	22	32	$C_{22}H_{45}O_2N_3$	10.96	10.84

[a] β-(N-Piperidyl)-ethyl. [b] β-(2-Pyridyl)-ethyl. [c] N-Piperidylmethyl.

dibutylamino and n was two. β-Diethylaminoethyl tributylcarbinylcarbamate, the most active member of the series, was twenty times more active than papaverine and possessed one-thirtieth the activity of atropine. In general, the structural requirements for antispasmodic activity observed in the quaternary carbon compounds described previously[5] apply to the dialkylaminoalkyl trisubstituted carbinylcarbamates.

Experimental

The synthesis of the trialkylcarbinylisocyanates has been described.[7] The dialkylaminoalkanols were obtained either from commercial sources or were prepared by standard procedures.

Dialkylaminoalkyl Trialkylcarbinylcarbamates.—The preparation of β-diethylaminoethyl tributylcarbinylcarbamate will illustrate the general method: A solution of 11.3 g. (0.05 mole) of tributylcarbinylisocyanate and 8.2 g. (0.07 mole) of β-diethylaminoethanol in 50 ml. of dry xylene was refluxed for 48–72 hours. The xylene was removed *in vacuo* and the residue was fractionated. After a small forerun, the carbamate was collected as a yellow, viscous oil. The methiodide melted at 118–118.5° after two recrystallizations from benzene–petroleum ether.

Anal. Calcd. for $C_{21}H_{45}O_2N_2I$: N, 5.78; I, 26.8. Found: N, 5.50; I, 27.2.

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(7) N. Sperber and R. Fricano, THIS JOURNAL, **71**, 3352 (1949).

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The Gentiobiose Heptaacetates

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Bergmann and W. Freudenberg[2] prepared a substance designated α-gentiobiose heptaacetate, m.p. 178° (cor.), $[\alpha]^{20}D$ + 35.1° → +31.6° (pyridine), from amygdalin. We wish to report the synthesis of β-gentiobiose heptaacetate, m.p. 113–115°, $[\alpha]^{28}D$ + 2.9° (chloroform), $[\alpha]^{28}D$ − 7.0° (initial, extrapolated) +34.1° (5 hr., pyridine), together with evidence that the "alpha" form previously reported is a crystalline molecular compound containing both anomers in a ratio $\alpha:\beta$ = 3:1. When held at a temperature of 135–140° the liquid beta isomer is converted to a crystalline solid which after recrystallization from suitable solvents appears to be identical with that reported by Bergmann and Freudenberg; m.p. 175–178°, $[\alpha]^{28}D$ + 36° (chloroform), $[\alpha]^{28}D$ + 35° (pyridine). The rotation data in pyridine indicate that this material is approximately an equilibrium mixture of the alpha and beta anomers. Applying Hudson's[3] rules of isorotation, the rotational value of α-gentiobiose heptaacetate can be calculated from values for β-gentiobiose heptaacetate and the anomeric forms of gentiobiose octaacetate.

α-Gentiobiose octaacetate, $A + B = 35{,}500$
β-Gentiobiose octaacetate, $-A + B = -3{,}600$
$2B = 31{,}900$

A represents the rotational contribution of the carbonyl group and B that of the remainder of the molecule.

α-Gentiobiose heptaacetate, $A' + B = x$
β-Gentiobiose heptaacetate, $-A' + B = 1{,}800$

Then it follows that the value of x, the molecular rotation of α-gentiobiose heptaacetate, is 30,100° ($[\alpha]D$ + 47.4°, chloroform). The molecular rotation of 23,000° ($[\alpha]D$ + 36.1°, chloroform) found for the equilibrium mixture would indicate the presence of approximately three parts of the alpha to one of the beta anomer. The specific reaction constant of the mutarotation in pyridine of the beta anomer was first order, indicating the presence of essentially only alpha and beta anomers in solution. However, X-ray powder diffraction diagrams showed that the material isolated from the equilibrium mixture contained no admixed crystalline β-gentiobiose heptaacetate. This equilibrium material must therefore be a crystal compound from the two isomers. Similar molecular compounds between anomers have been reported by Hockett and Hudson for methyl D-xyloside and for lactose.[4]

Experimental

β-Gentiobiose Heptaacetate.—Five grams of β-gentiobiose octaacetate was converted to heptaacetyl-α-gentiobiosyl bromide by the method of Zemplén[5]; yield 3.9 g.

(1) Corn Industries Research Foundation Associate of The Ohio State University Research Foundation (Project 203).

(2) M. Bergmann and W. Freudenberg, *Ber.*, **62**, 2783 (1929).

(3) C. S. Hudson, THIS JOURNAL, **31**, 66 (1909).

(4) R. C. Hockett and C. S. Hudson, *ibid.*, **53**, 4454, 4455 (1931).

(5) G. Zemplén, *Ber.*, **57**, 698 (1924).

The acetobromo derivative was dissolved in acetone[6] (50 ml.) and stirred overnight with 6 g. of silver carbonate. The solution was filtered and evaporated to a sirup under reduced pressure at 35°. The sirup crystallized immediately after solution in cool ethanol; yield 2.7 g., m.p. 111–115°. The dry material was covered with ethanol and allowed to stand overnight. When it was filtered and dried the constants were: m.p. 113–115°, $[\alpha]^{27}D$ + 2.9° (*c* 2.9, chloroform), $[\alpha]^{27}D$ − 7.0° (initial, extrapolated) → +34.1° (5 hr., *c* 3.1, pyridine) with *k* (first order) 0.01 (minutes and decimal logarithms). X-Ray powder diffraction data: 7.90[7]–25,[8] 7.16–20, 6.21–30, 5.54–20, 5.12–30, 4.72–70, 3.99–100, 3.54–15, 3.34–15, 3.10–5, 2.97–5, 2.71–10, 2.80–5, 1.93–5, 1.66–5. Further crystallization from acetone–ether–petroleum ether did not change these constants.

Anal. Calcd. for $C_{12}H_{15}O_{11}(CH_3CO)_7$: C, 49.05; H, 5.70; CH_3CO, 11.0 ml. 0.1 *N* NaOH per 100 mg. Found: C, 49.25; H, 5.66; CH_3CO, 11.0 ml.

Conversion of β-Gentiobiose Heptaacetate to the Alpha-beta Compound.—A sample of β-gentiobiose heptaacetate was heated in an oil-bath at 135° for 45 minutes. The resulting crystalline material was recrystallized from acetone–ether–petroleum ether; m.p. 175–177°, $[\alpha]^{25}D$ + 36.1° (*c*, 3.2, chloroform), $[\alpha]^{28}D$ + 34.7° (*c* 3.25, pyridine, no detectable mutarotation). Further recrystallizations did not change these constants. The above values are in substantial agreement with those of Bergmann and Freudenberg.[2] X-Ray powder diffraction data[7,8]: 5.50–80, 4.90–90, 4.47–100, 3.92–70, 3.57–50, 3.36–60, 3.12–5, 2.92–5, 2.80–20, 2.60–10, 2.03–10, 1.91–10, 1.74–10.

(6) E. Fischer and K. Hess, *Ber.*, **45**, 912 (1912).

(7) Interplanar spacing, Å., Cu K_α radiation.

(8) Relative intensity as percentage strongest line; estimated visually.

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The Rate of Absorption of and the Formation of Glycogen by DL-Homoserine as Compared with DL-Alanine[1]

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Homoserine, or α-amino-γ-hydroxybutyric acid, is believed to be one of the primary products of the action of cystathionase upon cystathionine. This hypothesis appears more likely to be so in view of the recent findings of Carroll, Stacy and du Vigneaud,[2] that homoserine, but not α-aminobutyric acid, is converted to α-ketobutyric acid by liver extracts which form cysteine and α-ketobutyric acid from cystathionine. This observation, together with the finding of McCoy, Meyer and Rose[3] that homoserine will not support the growth of rats on a threonine-deficient diet, and Armstrong and Binkley's[4] demonstration that the transulfuration reaction is not reversible, comprise the total knowledge of the metabolism of this compound in animals.

We have determined the rate of absorption of DL-homoserine from the intestine of the rat; similar measurements on DL-alanine were done for comparison. The glycogen contents of the livers of some of the rats used were also determined.

Method.—The DL-homoserine used was prepared from γ-butyrolactone by the method of Livak, *et al.*[5a,b] It was analyzed by the Kjeldahl method and by the carboxyl nitrogen method of Van Slyke, *et al.*,[6] and was found to be pure. The DL-alanine used was analyzed by the carboxyl nitrogen method, and was also pure.

In the first experiments, the amino acids were given by stomach tube to male white Sprague–Dawley rats which had been fasted for 24 hours. These rats had been kept in the laboratory for some weeks after their receipt from Sprague–Dawley, Inc., and were on the average larger than is desirable for studies of this kind. The rats weighed 130 to 330 g.; most of them were near 275 g. in weight. The doses of homoserine ranged from 193 to 273 mg./100 g. rat; the doses of alanine ranged from 185 to 288 mg./100 g. rat. The control rats were fed nothing. Three hours after the compounds were given, the rats were killed. Glycogen was determined in a portion of the liver by the method of Good, Cramer and Somogyi.[7]

For determination of the rate of absorption from the gut, gastro-intestinal tract and its contents were ground in a Waring blendor with 1% picric acid. The resulting slurry was diluted to 100 ml. with 1% picric acid in a volumetric flask and filtered through muslin, and carboxyl nitrogen was determined in aliquots of the picric acid solution.[8] Control experiments, in which homoserine and alanine were added to such gastrointestinal preparations showed that alanine was quantitatively recovered, but that recoveries of 94% were obtained for homoserine. A correction for this low recovery is incorporated in the values for homoserine absorptions reported here.

In the second set of experiments, the rats used were younger, smaller, and more carefully selected with respect to weight. Two absorption periods—two hours and one hour—were studied. Twelve rats which had been fasted for 48 hours were used for each experiment. Four rats served as controls; four were given DL-alanine, and four were given DL-homoserine. The analytical procedures were the same as those used in the first experiment, except that glycogen determinations were not done.

Results.—The results obtained for the rates of absorption are given in Table I.

TABLE I

RATES OF ABSORPTION OF DL-ALANINE AND DL-HOMOSERINE FROM THE INTESTINE OF THE RAT

Amino acid fed	Length of absorption period, hr.	Number of rats in group	Weight range of rats, g.	Dose range of amino acid, mg./100 g.	Rate of absorption, mg./100 g./hr.
DL-Alanine[a]	3	12	170–330	185–288	64 ± 4
DL-Homoserine[a]	3	11	230–310	193–273	60 ± 3
DL-Alanine[b]	2	4	115–135	198–211	94 ± 3
DL-Homoserine[b]	2	4	127–145	203–206	73 ± 2
DL-Alanine[c]	1	4	104–112	147–153	84 ± 4
DL-Homoserine[c]	1	4	100–112	150–153	68 ± 6

[a] The average total carboxyl N content of the gastrointestinal tracts of 11 control rats was 5.14 mg. by our procedure. This figure was used as a blank in calculating the absorption rates. [b] The average total carboxyl N content of the gastrointestinal tracts of 4 control rats was 3.80 mg. This figure was used as a blank. [c] The average total carboxyl N content of the gastrointestinal tracts of 4 control rats was 3.40 mg. This figure was used as a blank.

TABLE II

GLYCOGEN CONTENTS OF LIVERS OF RATS FED DL-HOMOSERINE AND DL-ALANINE

Group	Number of rats in group	Liver glycogen, %
Controls	9	0.26 ± 0.08
Alanine	10	.92 ± .05
Homoserine	11	.22 ± .05

(1) This work was supported in part by a grant from the American Cancer Society.

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(4) M. D. Armstrong and F. Binkley, *ibid.*, **174**, 889 (1948).

(5) (a) J. E. Livak, E. C. Britton, J. C. VanderWeele and M. F. Murray, THIS JOURNAL, **67**, 2218 (1945). (b) We wish to thank the Cliffs Dow Chemical Company for the generous gift of the necessary γ-butyrolactone.

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(8) P. B. Hamilton and D. D. Van Slyke, *ibid.*, **150**, 231 (1943).