bution to χ_1. With the pure polymer and at 40° the entropy for acetone (beyond the configurational) is approximately −2.5 e.u. and for propanol it is approximately 6 e.u. Thus for both systems the data indicate that the entropy contribution to χ_1 is comparable in size to the contribution from the heat of mixing. For both systems the entropy and heat terms give opposing contributions to χ_1, a result which is in accord with an early discussion by Huggins.[11]

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The Sakaguchi and Biacetyl Reactions for the Identification of Alkyl Guanidines

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Some representative mono-, di- and trialkyl-substituted guanidines have been prepared and their reactivity to the Sakaguchi[1] and biacetyl[2] tests has been determined (Table I). Of the compounds tested, only methylguanidine, in which the grouping $RNHC(=NH)NH_2$ is present, gives a positive Sakaguchi test. Guanidine and the di- and trialkyl substituted compounds do not react even at levels 100-fold as great. These findings are contrary to the report of Poller[3] who maintained that the symmetrically substituted di- and trialkyl compounds give positive reactions. Since this worker failed to characterize adequately his test compounds, it is impossible to determine whether these were authentic.

The most intense coloration in the biacetyl reaction for this series of compounds is given by *asym*-N,N-dimethylguanidine, in which the grouping $RR'NC(=NH)NH_2$ is present. The compounds in which one or both of these alkyl groups are replaced by hydrogen show lower intensities and develop their color at a slower rate. Compounds in which two or three of the nitrogen atoms are substituted with alkyl groups fail to react even at levels 2000-fold as great.

The alkyl guanidines used in this work were prepared by established methods already described in the literature. They were identified by m.p., elementary analysis and alkaline hydrolysis which produced carbon dioxide and volatile amines in the theoretical amounts.[4] The volatile amines were identified by paper chromatography.[5] Since these well-characterized compounds were available, potentiometric titrations were carried out to determine their base strengths. All of these compounds were found to have dissociation constants greater than pK_a 11.4 and the titrations did not differ within the experimental accuracy from the values for a water blank. These results dispute the earlier findings of Davis and Elderfield[6] that symmetrically substituted dialkyl guanidines are weaker bases than other types of alkyl substituted guanidines. The present work is in agreement with the findings of Neivelt, *et al.*,[7] and of Angyal and Warburton.[8]

TABLE I

SAKAGUCHI AND BIACETYL REACTIONS OF ALKYL GUANIDINES

Compound	Sakaguchi reaction[a] Amt. tested, μg.	Sakaguchi reaction[a] Intensity of color[c]	Biacetyl reaction[b] Amt. tested, μg.	Biacetyl reaction[b] Intensity of color[d]
Guanidine	100	0	6	1.0
Methylguanidine	630	1600	5	1.0
asym-N,N-Dimethylguanidine	362	0	0.5	1.0
sym-N,N′-Dimethylguanidine	414	0	1000	0
sym-N,N′-Diethylguanidine	100	0	1000	0
sym-N,N′-Dibutylguanidine	100	0	1000	0
sym-N,N′,N″-Triethylguanidine	100	0	1000	0

[a] The procedure used was identical to that described by A. A. Albanese and J. E. Frankston, *J. Biol. Chem.*, **159**, 185 (1945), with the exception that 3.5 min. was allowed after the addition of the hypochlorite reagent instead of 1.0 min. as used by these authors. [b] This reaction was carried out according to the directions of P. Eggleton, S. R. Elsden and N. Gough, *Biochem. J.*, **37**, 526 (1943). [c] Known concentrations of arginine monohydrochloride (Eastman Kodak Co. Anal.) were used to standardize the procedure. Color intensities were determined with a Coleman colorimeter using a 530 mμ filter. The intensity is given in terms of μg. of arginine base. The minimum amount of arginine that could be detected was 1 μg. [d] Color intensities were determined by comparison with the color produced by known concentrations of creatine hydrate (Eastman Kodak). The intensity is given in terms of μg. of creatine hydrate. The minimum amount of creatine hydrate that could be detected was 0.5 μg.

Experimental[9]

Guanidine Hydrochloride (Eastman Kodak Co.).—*Anal.* Calcd. for CH_6N_3Cl: N, 44.00. Found: N, 44.52, 44.26.

Methylguanidine Sulfate.—This compound was prepared[10] from dicyanodiamide and methylamine hydrochloride by the procedure of Traube and Gorniak,[11] m.p. 238° (lit. m.p. 238°,[11] 239–240°,[12] 240°,[8] 238–239°[6a]).

Anal. Calcd. for $C_4H_{16}N_6SO_4$: C, 19.67; H, 6.60. Found: C, 19.64, 19.67; H, 6.55, 6.55.

***asym*-N,N-Dimethylguanidine Sulfate.**—S-Methylisothiourea sulfate was treated with aqueous dimethylamine according to the procedure of Phillips and Clarke,[12] m.p. 278–279° (lit. m.p. 285–288°,[12] 295–297°,[8] 285–287°[6a]).

Anal. Calcd. for $C_6H_{20}N_6SO_4$: C, 26.46; H, 7.40. Found: C, 26.32, 26.47; H, 7.20, 7.35.

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sym-N,N'-**Dimethylguanidine Hydrobromide.**—The preparation of this compound was patterned after the procedure of Schenck.[13] This involved the reaction of 45 ml. of a 10% solution of methylamine (0.14 mole) in absolute ethanol with 5.2 g. of cyanogen bromide[14] (0.05 mole) at room temperature for 48 hours in a tightly stoppered flask. Evaporation of the contents of the flask *in vacuo* yielded the crude hydrobromide, m.p. 142°. After four recrystallizations from a 1:1 ether–absolute ethanol mixture at 6°, the purified product melted at 144°.

Anal. Calcd. for $C_3H_{10}N_3Br$: C, 21.44; H, 5.99. Found: C, 21.53, 21.42; H, 5.88, 5.95.

The picrate was prepared, m.p. 177° (lit. m.p. 177.0, 177.5°,[7] 178°[13]).

sym-N,N'-**Diethylguanidine Picrate.**—*sym*-N,N'-Diethylguanidine hydrobromide was prepared from ethylamine and cyanogen bromide in a manner similar to that described above for the corresponding dimethyl compound. This compound failed to crystallize when treated in the usual manner and was converted to the picrate, m.p. 143–144° (lit. m.p. 143–144°,[7] 141°[15]).

Anal. Calcd. for $C_{11}H_{16}N_6O_7$: C, 38.37; H, 4.68. Found: C, 38.13, 38.18; H, 4.79, 4.55.

sym-N,N'-**Dibutylguanidine Picrate.**—*sym*-N,N'-Dibutylguanidine hydrobromide was prepared from *n*-butylamine and cyanogen bromide in a manner similar to that described for the corresponding dimethyl compound. This compound failed to crystallize when treated in the usual manner and was converted to the picrate, m.p. 120–122° (lit. m.p. 122.5°[16]).

Anal. Calcd. for $C_{15}H_{24}N_6O_7$: C, 44.99; H, 6.04. Found: C, 44.91; H, 5.94.

sym-N,N',N''-**Triethylguanidine Hydroiodide.**—This compound was prepared by a procedure adapted from the method of Angyal and Warburton[8] for the preparation of the symmetrical trimethyl compound. One mole of ethyl isothiocyanate (Eastman Kodak Co.) was treated with 1.2 moles of ethylamine in ether solution to give N,N'-diethylthiourea, m.p. 77° (lit. m.p. 77°). This compound (1 mole) was treated with ethyl iodide (1 mole) to give S-ethyl N,N'-diethylthiouronium iodide which was isolated by evaporation *in vacuo* and allowed to react with 2 moles of ethylamine in aqueous solution for 24 hours on the water-bath and a further 24 hours under reflux. The product, *sym*-N,N',N''-triethylguanidine hydroiodide, was isolated by evaporating the solution to dryness *in vacuo*. Purification was accomplished by crystallization from ethanol–ether solution or by crystallization from water at 5°. The purified product melted at 139°.

Anal. Calcd. for $C_7H_{18}N_3I$: C, 31.00; H, 6.69. Found: C, 31.90, 31.06; H, 6.54, 6.37.

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The Synthesis of α,γ-Dihydroxy-β-amino-*n*-butyric Acid

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The structural formula of the base sphingosine is now known in sufficient detail to recognize that the degradation of this base *via* ozonolysis should give as one of the degradation products an α,γ-dihydroxy-β-amino-*n*-butyric acid instead of an α-amino-β,γ-dihydroxy-*n*-butyric acid as was originally claimed by Klenk and Diebold.[3,4] Although it was shown by Niemann and Nichols,[5] through the synthesis of D-*erythro*- and D-*threo*-α-amino-β,γ-dihydroxy-*n*-butyric acid, that Klenk and Diebold's acid could not possess the structure claimed for it there appears to be no record of an attempt to synthesize the acid which Klenk and Diebold isolated. Therefore we wish to describe in this communication a synthesis of an optically inactive α,γ-dihydroxy-β-amino-*n*-butyric acid by a method which should be capable of application to the synthesis of the various optically active forms.

α-Bromo-β-ethoxypropionic acid (I) was prepared essentially as described by Wood and du Vigneaud.[6] Ammonolysis of I gave α-amino-β-ethoxypropionic acid (II) which was condensed with phthalic anhydride to give α-phthalimido-β-ethoxypropionic acid (III). All attempts to prepare an ester of III by the direct condensation of an ester of I with potassium phthalimide were unsuccessful. The reaction of III with thionyl chloride gave the corresponding acid chloride IV which was allowed to react with diazomethane to give α-phthalimido-β-ethoxyethyl diazomethyl ketone (V). Rearrangement of V with silver oxide in methanol gave methyl β-phthalimido-γ-ethoxy-*n*-butyrate (VI) which was hydrolyzed to the corresponding acid VII. Bromination of VII, in the presence of red phosphorus, gave a mixture of isomeric α-bromo-β-phthalimido-γ-butyrolactones (VIIIa and VIIIb) which were separated on the basis of their different solubilities in ethanol. When the less soluble and higher melting isomer VIIIb was heated under refluxing conditions with either silver or sodium acetate in acetic acid VIIIb was recovered unchanged. After preliminary experiments had shown that α-bromo-β-phthalimidopropionic acid (IX) could be converted into isoserine by heating IX under refluxing conditions with an aqueous suspension of barium carbonate, VIIIb was similarly treated to give a DL-mixture of one of the diastereoisomeric forms of α,γ-dihydroxy-β-amino-*n*-butyric acid.

Experimental[7,8]

α-**Amino-β-ethoxypropionic Acid (II).**—Ethyl α-bromo-β-ethoxypropionate[6] (60 g.) was converted into II essentially as described by Wood and du Vigneaud.[6] Evaporation of the ammoniacal reaction mixture to dryness followed by extraction of the resulting residue with a large volume of boiling ethanol gave 19.35 g. of II, colorless platelets, m.p. 238–240° with decomposition.

Anal. Calcd. for $C_5H_{11}O_3N$ (133.2): C, 45.1; H, 8.3; N, 10.5. Found: C, 45.2; H, 8.3; N, 10.4.

α-**Phthalimido-β-ethoxypropionic Acid (III).**—An intimate mixture of 19.3 g. of II and 21.5 g. of phthalic anhydride was heated to 150–160°. When the reaction had subsided the reaction mixture was cooled, dissolved in hot benzene, the solution cooled, the brown crystalline precipitate recovered, dissolved in hot aqueous ethanol, the hot solution decolorized with Norite, and the solution cooled to give 19.7 g. of III, m.p. 136–138°.

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