activity.[2] However, it is worth noting that much higher concentrations of thrombin are required to hydrolyze LEe than are needed to hydrolyze TAMe[3] or to activate fibrinogen[8] at the same *p*H. It is interesting to speculate that, while the primary action of thrombin on fibrinogen may be toward arginyl bonds, a slower secondary action may take place at lysyl bonds. If so, the latter activity may possibly be the origin of the phenomenon reported by Guest and Ware[5] wherein purified thrombin at very high concentration caused the lysis of fibrin clots, an action which was not prevented by STI.

In support of this additional specificity for thrombin we may cite some preliminary experiments involving Seegers' citrate thrombin and the oxidized B chain of insulin, prepared as described elsewhere.[9] Thrombin (50–100 TAMe units ml.) was incubated with oxidized B-chain (4–8 mg./ml.) at *p*H 8 in 0.1 *M* ammonium acetate for 18–24 hr. at 25°. In some experiments STI was added (0.8 mg./ml.) Parallel experiments were carried out using trypsin (0.1–0.2 mg./ml) in place of thrombin. The hydrolysis of the lysyl-alanine bond of the B-chain was assessed by detection of the alanine fragment by paper chromatography of the free amino acid and also of its DNP-derivative. With either method the intensity of the spot was undiminished by the presence of STI when thrombin was used rather than trypsin. No quantitative data are yet available for the degree of hydrolysis of the lysyl-alanine bond. Qualitatively, it appeared that thrombin was 10–20% as effective as trypsin under the conditions stated.

The activity of thrombin toward the arginyl-glycine bond of the B-chain is still under investigation. Further studies are also being carried out on the activity of thrombin toward synthetic lysyl substrates. We are indebted to Dr. W. H. Seegers for his generous gifts of purified thrombin.

(8) E. Mihalyi, quoted by J. A. Gladner and K. Laki, *Arch. Biochem. and Biophys.*, **62**, 501 (1956).

(9) S. J. Leach and H. A. Scheraga, *Compt. rend. Lab. Carlsberg*, in press.

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RECEIVED OCTOBER 14, 1957

THE AZEOTROPE OF MONOCHLORODIFLUOROMETHANE AND DICHLORODIFLUOROMETHANE

Sir:

There is an azeotrope of monochlorodifluoromethane and dichlorodifluoromethane. That these two common refrigerants form an azeotrope has not been generally recognized, since the feasibility of their separation by simple distillation has been tacitly assumed in both the technical and patent literature in various instances. Furthermore, the azeotrope may occur in practical refrigeration systems, since dichlorodifluoromethane is sometimes added to monochlorodifluoromethane, when the latter is used as a refrigerant, in order to improve the low-temperature solubility of lubricating oil.

The existence of the azeotrope was demonstrated in two ways.

Reflux boiling points were measured, Table I, showing a minimum at about −41.4°, only 0.6° below the boiling point of monochlorodifluoromethane, at a composition of about 25% dichlorodifluoromethane by weight. There is little change in boiling point between 10 and 50% dichlorodifluoromethane by weight, the values lying between about −41.0 and −41.4°.

The existence of the azeotrope was confirmed by fractionating a mixture of 58.2% dichlorodifluoromethane and 41.8% monochlorodifluoromethane at high reflux in a low-temperature Podbielniak still (Cat. No. 407). Portions of the constant-boiling distillate analyzed 24.5 to 26.7% dichlorodifluoromethane by weight on the basis of the density of the gas and 25 to 29% dichlorodifluoromethane by weight based on infrared absorption. These results are in accord with expectation from the boiling-point data.

TABLE I

NORMAL BOILING POINTS OF MIXTURES OF MONOCHLORODIFLUOROMETHANE AND DICHLORODIFLUOROMETHANE

Weight % dichlorodifluoromethane in mixture	Boiling point °C.	Weight % dichlorodifluoromethane in mixture	Boiling Point °C.
0.0	−40.80	51.6	−40.73
1.4	−40.76	53.9	−40.63
2.9	−40.80	57.5	−40.54
5.2	−40.94	58.4	−40.54
6.9	−40.89	64.2	−39.84
9.0	−40.99	69.9	−38.93
15.0	−41.31	73.1	−38.33
21.9	−41.41	78.4	−37.14
27.8	−41.41	83.9	−35.87
32.5	−41.39	89.4	−34.26
38.8	−41.39	100.0	−29.80
44.4	−40.93		

"FREON" PRODUCTS LABORATORY
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RECEIVED SEPTEMBER 16, 1957

STUDIES ON POLYPEPTIDES. XI. PREPARATION OF AN OCTAPEPTIDE POSSESSING MELANOCYTE-STIMULATING ACTIVITY[1]

Sir:

Structural studies of the corticotropins[2–5] and of the melanocyte-stimulating hormones (α- and β-M.S.H.)[6–8] have shown that the molecules of these substances contain a common amino acid sequence ("core") possessing the structure met-glu-his-phe-arg-try-gly. Since all these hormones stimulate

(1) Supported by grants from the U. S. Public Health Service, the National Science Foundation, Armour and Company, and Eli Lilly and Company.

(2) P. H. Bell, THIS JOURNAL, **76**, 5565 (1954).

(3) W. F. White and W. A. Landmann, *ibid.*, **77**, 1711 (1955).

(4) R. G. Shepherd, S. D. Willson, K. S. Howard, P. H. Bell, D. S. Davies, S. B. Davis, E. A. Eigner and N. E. Shakespeare, *ibid.*, **78**, 5067 (1956).

(5) C. H. Li, I. I. Geschwind, R. D. Cole, I. D. Raacke, J. I. Harris and J. S. Dixon, *Nature*, **176**, 687 (1955).

(6) J. I. Harris and A. B. Lerner, *ibid.*, **179**, 1346 (1957).

(7) J. I. Harris and P. Roose, *ibid.*, **178**, 90 (1956).

(8) I. I. Geschwind, C. H. Li and L. Barnafi, THIS JOURNAL, **78**, 4494 (1956).

melanocytes, it was suggested[6-8] that the essential minimum structural requirement for melanocyte-stimulating activity may reside in this common sequence. We have completed recently a synthesis of the octapeptide serylmethionylglutaminylhistidylphenylalanylarginyltryptophylglycine (7-L) and have tested its ability to stimulate melanocytes. The present communication summarizes our findings. Carbobenzoxy-L-methionine was coupled with L-glutamine to give carbobenzoxy-L-methionyl-L-glutamine, m.p. 159–161°, $[\alpha]^{25}D$ −13.6° (in 95% ethanol). *Anal.* Calcd. for $C_{18}H_{25}O_6N_3S$: C, 52.5; H, 6.1; N, 10.2; S, 7.8. Found: C, 52.6; H, 6.1; N, 10.3; S, 7.6, which was decarbobenzoxylated to L-methionyl-L-glutamine, m.p. 220–221°, $[\alpha]^{25}D$ +14.1° (in 10% ammonia), R_f = 0.43 (Partridge), R_f = his$^+$ (2-butanol–ammonia). *Anal.* Calcd. for $C_{10}H_{19}O_4N_3S$: N, 15.2. Found: N, 15.6. Treatment with carbobenzoxy-L-serine azide converted the dipeptide into carbobenzoxy-L-seryl-L-methionyl-L-glutamine, m.p. 172–173°, $[\alpha]^{24}D$ −24.7° (in 95% ethanol). *Anal.* Calcd. for $C_{21}H_{30}O_8N_4S$: C, 50.6; H, 6.1; N, 11.2. Found: C, 50.3; H, 5.9; N, 11.6, which was converted into the hydrazide, m.p. 211–212°. *Anal.* Calcd. for $C_{21}H_{32}O_7N_6S$: N, 16.4. Found: N, 16.1. The corresponding azide was then coupled in N,N-dimethylformamide with the triethyl ammonium salt of L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine[9] to give carbobenzoxyserylmethionylglutaminylhistidylphenylalanylarginyltryptophylglycine, R_f = 0.79 (Partridge). Single spot, positive reaction with the Pauly, Sakaguchi, Ehrlich and methionine reagents,[4] ninhydrin negative. The acylated octapeptide was decarbobenzoxylated and the ensuing free octapeptide purified by chromatography on cellulose. The purified material gave a single spot, R_f = 0.48 (Partridge) regardless of whether the papers were sprayed with the ninhydrin, Pauly, Sakaguchi, Ehrlich or methionine reagents. The peptide was completely digestible with leucine aminopeptidase[10] and quantitative amino acid analyses of the digest revealed the presence of an equimolar mixture of the expected amino acids. Other ninhydrin positive substances were not seen on the chromatograms. These results establish the stereochemical homogeneity of the compound. The ability of the octapeptide and of its carbobenzoxy derivative to stimulate melanocytes was determined,[11] and both compounds exhibited an activity of 0.7×10^6 M.S.H. units per gram.

These results demonstrate that the glutamine analog of a peptide, possessing an amino acid sequence corresponding to the "core" common to the corticotropins and the melanocyte expanding hormones, is indeed endowed with melanocyte-stimulating activity, but that this activity is of a low order of magnitude compared to that of the intact hormones. It is of interest to note that substitution of the N-terminal amino group of the octapeptide by the bulky carbobenzoxy group does not destroy the biological activity.

(9) K. Hofmann, M. E. Woolner, G. Spühler and E. T. Schwartz, THIS JOURNAL, in press.

(10) D. H. Spackman, E. L. Smith and D. M. Brown, *J. Biol. Chem.*, **212**, 255 (1955).

(11) We wish to express our sincere appreciation to Drs. M. R. Wright and A. B. Lerner, Department of Medicine, Yale University School of Medicine, for these determinations.

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RECEIVED SEPTEMBER 9, 1957

THE REDUCTION OF TRIPHENYLACETONITRILE BY THE α-HYDROGEN ATOM OF BENZYLMAGNESIUM CHLORIDE

Sir:

Certain reactions of nitriles with Grignard regents have been best explained[1] by the assumption of the exchange of nitrile and magnesio chloride groups

$$RCN + R'MgCl \longrightarrow R'CN + RMgCl \quad (1)$$

In what has appeared to be "the most clear-cut example reported,"[1] treatment of triphenylacetonitrile (I) first with benzylmagnesium chloride and then with water produced triphenylmethane (II, equation 2).[2]

$$\underset{\text{I}}{(PH)_3CCN} \xrightarrow[(2)\ H_2O]{(1)\ PhCH_2MgCl} \underset{\text{II}}{(Ph)_3CH} \quad (2)$$

If exchange occurs, the reactions producing triphenylmethane could be[1]

$$(PH)_3CCN + PhCH_2MgCl \longrightarrow \underset{\text{III}}{(Ph)_3CMgCl} + \underset{\text{IV}}{PhCH_2CN} \quad (3)$$

$$(Ph)_3CMgCl + H_2O \longrightarrow (Ph)_3CH + Mg(Cl)(OH) \quad (4)$$

Although equations 3 and 4 seem to be a reasonable explanation[1] of reaction 2, they are now invalidated by the following findings: (1) When the proposed intermediates, triphenylmethylmagnesium chloride (III) and phenylacetonitrile (IV), are mixed and then treated with water the yield of triphenylmethane is negligible. (2) When the reaction between triphenylacetonitrile and benzylmagnesium chloride is run as described[2] and the reaction mixture is then treated with excess carbon dioxide before hydrolysis no triphenylacetic acid is formed. (3) If the reaction mixture of triphenylacetonitrile and benzylmagnesium chloride is hydrolyzed with water labeled with tritium, no tritium appears in the triphenylmethane. However, when the reaction is carried out with benzylmagnesium chloride labeled on the α-carbon atom with tritium, tritium does appear in the triphenylmethane. The transfer of tritium takes place with an isotope effect of approximately five. Thus *the source of hydrogen found ultimately in the triphenylmethane (II, equation 2) is the benzyl grouping in the original Grignard reagent.*

A possibility for the course of this reaction is shown in equation 5. Once the complex (V) between nitrile and Grignard reagent is formed,

(1) M. S. Kharasch and O. Reinmuth, "Grignard Reactions of Nonmetallic Substances," Prentice-Hall, Inc., New York, N. Y., 1954, pp. 779–782.

(2) Ramart-Lucas and Salmon-Legagneur, *Bull. soc. chim.*, (4) **43**, 321 (1928). Ramart-Lucas isolated the hydrocarbon (II) in 70% yield from the nitrile (I) by using excess Grignard reagent in boiling toluene. By analysis through isotopic dilution we find II formed to the extent of 97% in the toluene solvent at 70°.