mixture formed by *S. chrysomallus* when 0·5 per cent DL-valine was added to a glycerol–nitrate medium[5].

In the course of an investigation of the biosynthesis of the actinomycins produced by a strain of *S. antibioticus*, we have made observations regarding the marked influence of suitable amino-acids upon the relative amount of any one of the naturally produced actinomycins. The technique employed has been the following. Washed cells of the organism, previously grown in *N–Z* amine medium for 48 hr. on a rotary shaker at 28° C., were inoculated into a chemically defined medium composed of (per cent): D-galactose, 1·0; L-glutamic acid, 0·2; K_2HPO_4, 0·1; $ZnSO_4 \cdot 7H_2O$, 0·0025; $MgSO_4 \cdot 7H_2O$, 0·0025; $CaCl_2 \cdot 2H_2O$, 0·0025; $FeSO_4 \cdot 7H_2O$, 0·0025; distilled water, *p*H 7·2–7·3. When production of actinomycin began (1–2 days) a given concentration of an amino-acid (0·001–0·25 per cent) was added to a number of flasks of the growing culture. After an additional production period of three to seven days, the actinomycin mixture synthesized was harvested and the actinomycins present were separated by means of a circular paper chromatographic technique previously described[7]. The actinomycins separate as yellow zones on a papergram, thus facilitating their detection. The relative percentages of components in a mixture were determined by a spectrophotometric method[3].

As revealed in Table 1, actinomycin-IV represents the major component of the actinomycin complex produced by *S. antibioticus* in the glutamic acid medium. Addition to the medium of one or another amino-acid, for example, hydroxy-L-proline, sarcosine N-acetylglycine, or L-*iso*leucine, brought about significant quantitative changes in the actinomycin complex synthesized. Components normally produced in small or even trace amounts were found, under the specific conditions now employed, to represent major constituents of such mixtures. Moreover, not only was the nature of the amino-acid important, but also the level added to growing cultures was significant in bringing about the changes observed. Results obtained in an experiment using different concentrations of sarcosine are presented in Table 2.

The precise role of an amino-acid in modifying the biosynthetic reactions leading to actinomycin formation is unknown at the present time. The actinomycins represent a group of chromopeptides[8,9], and, therefore, an amino-acid might be incorporated directly into certain actinomycin peptides or be

Table 1. INFLUENCE OF AMINO-ACIDS ON ACTINOMYCIN SYNTHESIS

	Medium	Concentration of amino-acid added (per cent)	Relative percentage of actinomycin components					
			I	II	III	IV	V	*
1	Glutamic acid medium	0·0	6·4	2·3	3·2	68·3	17·2	2·6
2	Glutamic acid medium + hydroxy-L-proline	0·25	31·0	3·8	7·1	25·3	30·0	3·0
3	Glutamic acid medium + sarcosine	0·05	9·1	25·6	35·0	24·4	5·9	0·0
4	Glutamic acid medium + N-acetylglycine	0·25	8·1	2·0	3·2	32·7	51·1	3·0
5	Glutamic acid medium + L-*iso*leucine	0·25	4·7	2·8	5·8	17·2	30·6	38·9

* Denotes an unidentified component which moves faster than component V in circular paper chromatography.

Table 2. INFLUENCE OF SARCOSINE CONCENTRATION ON ACTINOMYCIN SYNTHESIS

Concentration of sarcosine added (per cent)	Relative percentage of actinomycin components				
	I	II	III	IV	V
0·0	6·2	2·1	2·8	80·4	8·4
0·001	5·3	9·2	11·9	63·5	10·1
0·005	6·5	21·2	30·8	35·9	5·6
0·05	9·1	25·6	35·0	24·4	5·9

serving as a precursor for one naturally produced in limited amounts. Possibly it may interfere with the incorporation of an amino-acid into certain peptides, thereby inhibiting their formation and enabling greater synthesis of others instead[5]. Additional studies are necessary to learn how an amino-acid influences the reactions concerned with formation of this group of antibiotics.

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A New Synthesis of the Pentapeptide L-Histidyl-L-Phenylalanyl-L-Arginyl-L-Tryptophyl-Glycine and its Melanocyte-stimulating Activity

A SYNTHESIS of the pentapeptide H.His-Phe-Arg-Try-Gly.OH along classical lines has recently been described by Hofmann *et al.*[1]. The amino-acid sequence of this peptide, which occurs in adrenocorticotropic (ACTH) and melanocyte-stimulating (MSH) hormones[2], presents a number of considerable difficulties for chemical synthesis, chiefly owing to the presence of tryptophan, arginine and histidine. In this communication, we wish to report the synthesis of this pentapeptide in a stepwise manner beginning at the C-terminus with the *p*-(*p'*-methoxy-phenylazo)-benzyloxy carbonyl group (MZ group), described by one of us[3], used as the amino-protecting agent. In addition, the *p*-toluenesulphonyl (tosyl) group was introduced to protect the guanidino group of arginine, since this protecting group resists catalytic hydrogenation and is easily removed by sodium in liquid ammonia. When the peptide was assayed either in the hypophysectomized frog or on frog skin *in vitro*, it was found to possess melanocyte-stimulating activity.

Cyanomethyl carbobenzoxy L-tryptophanate[4] was condensed with methyl glycinate to give Z.Try-Gly.OCH_3. The dipeptide ester resulting from catalytic hydrogenation in the presence of palladium

was condensed with N^{α}-MZ-N^{G}-tosyl-L-arginine [prepared by treating MZ-L-arginine[3] with tosyl chloride, or by tosylating carbobenzoxy-L-arginine, removing the carbobenzoxy group and treating the N^{G}-tosyl arginine (R_F=0·36 in system *A*) with MZ-chloride; the product, N^{α}-MZ-N^{G}-tosyl-L-arginine: m.p. 115° C.; $[\alpha]_D = +6·1$, 1 per cent in CH_3COOH; $R_F^A = 0·61$ (system *A*), 0·38 (system *B*)] by means of reaction with dicyclohexyl carbodiimide (DCCI)[5], to give the amorphous MZ.Arg(N^{G}-tos)-Try-Gly-OCH_3, the R_F^A of which equals 0·70 in system *A*, 0·26 in system *B*, and 0·63 in system *C*. (R_F^A designates R_F values on the fully acetylated paper of Schleicher and Schuell. System *A* is *n*-BuOH/CH_3COOH/H_2O, 4 : 1 : 1; *B* is the lower phase of CCl_4/CH_3OH/pyridine/water, 4 : 2 : 1 : 1; *C* is the upper phase of this system. *D* is the system *n*-BuOH/pyridine/CH_3COOH/H_2O, 30 : 20 : 6 : 24; R_F values were obtained with Whatman No. 1 paper.) Catalytic reduction with 10 per cent palladium on charcoal in neutral solution released colourless H.Arg(N^{G}-tos)-Try-Gly-OCH_3, $R_F^A = 0·76$ in system *A* and 0·89 in system *D*, which gave a positive reaction with ninhydrin and the Ehrlich reagent. This compound was combined with MZ-L-phenylalanine[3] by means of reaction with DCCI, and the product was purified in a column of acetylated cellulose (system *C*). MZ.Phe-Arg(N^{G}-tos)-Try-Gly.OCH_3 was obtained in crystalline form: m.p. 130° C., $[\alpha]_D = -9·0°$, 1 per cent in CH_3COOH, $R_F^A = 0·36$ (system *C*). The absorption spectrum indicates 3 peaks at 290, 350 and 430 mμ with *E* 9,700, 24,700 and 4,500 respectively (ethanolic solution). H.Phe-Arg(N^{G}-tos)-Try-Gly.OCH_3, obtained by catalytic hydrogenation as a colourless glassy product (R_F = 0·84, system *A*), was further condensed with N^{α}-MZ-N^{Im}-benzyl-L-histidine, giving MZ.His(N^{Im}-benzyl)-Phe-Arg(N^{G}-tos)-Try-Gly.OCH_3 in the form of yellow microscopic platelets, m.p. 132° C., $[\alpha]_D$ −6·0, 1 per cent in CH_3COOH, $R_F^A = 0·19$ (system *B*); the spectrum shows 3 peaks at 290, 350 and 432 mμ with *E* 11,700, 31,900 and 1,640 respectively. Hydrogenation over 10 per cent palladium on charcoal and saponification with barium hydroxide yielded H.His(N^{Im}-benzyl)-Phe-Arg(N^{G}-tos)-Try-Gly.OH: R_F = 0·68 (system *A*) and 0·92 (system *D*). Treatment of this product in dry liquid ammonia at −30° C. with 20 equivalents of sodium for a short time, conversion of the resulting peptide to dihydrochloride, and extraction into glacial acetic acid gave the dihydrochloride of H.His-Phe-Arg-Try-Gly.OH as a colourless glassy product, R_{His} = 1·92 (system *A*), which gave a positive reaction with ninhydrin and the Pauly, Sakaguchi and Ehrlich reagents; amino-acid analysis of the peptide by the Levy method[6] and by the spectrophotometric procedure of Goodwin and Morton[7] gave the molar ratio: His/Phe/Arg/Try/Gly = 0·9/1·1/1·1/1·0/1·0. The product was completely split by trypsin at *p*H 7 to give H-His-Phe-Arg-OH (R_F = 0·11, system *A*) and H-Try-Gly-OH (R_F = 0·42, system *A*); reaction with chymotrypsin at *p*H 9 resulted in total cleavage of the peptide to H.His-Phe.OH, (R_F = 0·11, system *A*), H.Arg-Try.OH (R_F = 0·16, system *A*) and glycine (R_F = 0·23, system *A*).

The synthetic pentapeptide, when assayed for melanocyte-stimulating activity both on isolated frog skin[8] and in hypophysectomized *Rana pipiens*, was found to possess an activity of 3 × 10^4 MSH units per gm. A hexapeptide, H-Glu-His-Phe-Arg-Try-Gly.-OH, synthesized by H. Kappeler in the laboratory of one of us (R. S.) at Basle by conventional coupling of Glu, His-Phe-Arg, and Try-Gly, has been assayed for MSH activity and found to be more active (2 × 10^5 units per gm.) than the pentapeptide herein reported. It is of interest to note that Hofmann *et al.*[9] reported the synthesis of an octapeptide, H.Ser-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly.OH, with an MSH activity of 7 × 10^5 units per gm. When solutions of the synthetic product (1 mgm./ml.) in 0·1 *M* sodium hydroxide were kept at 100° C. for 15 min., the resulting products exhibited the characteristic 'prolongation' activity in hypophysectomized frogs found in MSH and ACTH preparations[2] that have been treated in a similar manner. Analysis by the Levy procedure[6] of the pentapeptide after alkali-heat treatment showed that the arginine residue has been partially converted into ornithine. (Earlier preliminary studies with I. I. Geschwind using MSH, ACTH and crude peptide preparations gave similar conclusions.) Whether or not the 'prolongation' effect is due solely to the modification of arginine in the peptide remains to be investigated. Further studies of these problems are in progress.

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Determination of Indole Acetic Acid by the Salkowsky Reaction

Recently attention has been focused on the reaction of indole acetic acid with ferric ions. Cohen *et al.*[1] have shown that indole acetic acid forms a chelate with iron at acid *p*H and this has been confirmed by Recaldin and Heath[2]. The latter state that at *p*H 2·6 iron slowly decomposes the indole acetic acid in the solution. The oxidation of indole acetic acid by ferric salts is the basis of the colorimetric determination of the acid based on the Salkowsky reaction[3–5]. It is generally supposed that these reactions measure the formation of a coloured oxidation compound of indole acetic acid formed at very acid *p*H in the presence of ferric chloride.

Chance observation in this laboratory (by Blumenthal–Goldschmidt) showed that the usual Tang–Bonner reagent gives a different and stronger colour