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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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]

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

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

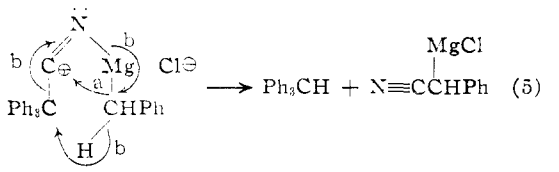
Although equations 3 and 4 seem to be a reasonable explanation[1] of reaction 2, they are now invalidated by the following findings: (1) When the proposed intermediates, triphenylmethylmagnesium chloride (III) and phenylacetonitrile (IV), are mixed and then treated with water the yield of triphenylmethane 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 Non-metallic 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°.

there occurs both the commonly accepted[3] 1,3-shift of the benzyl group from magnesium to nitrile-carbon (arrow a) and a concerted shift of hydrogen and electrons around a six-membered quasi-ring (arrows b). If these are the transformations which occur, phenylacetonitrile (from VI)



V II VI

should be isolable after hydrolysis. When the reaction was carried out with triphenylacetonitrile labeled in the nitrile group with carbon-14, labeled phenylacetonitrile was isolated after hydrolysis.[4,5]

(3) C. G. Swain, THIS JOURNAL, **69**, 2306 (1947); see also pp. 767–769 in ref. 1.

(4) In this run isotopic dilution showed that the phenylacetonitrile was formed in 35% yield. All remaining carbon-14 was found in a non-volatile, amorphous, organic material formed presumably by polymerization of VI; *cf.* A. Rondou, *Bull. soc. chim. Belg.*, **31**, 231 (1922).

(5) The authors wish to acknowledge the many helpful discussions of this work with Dr. C. J. Collins.

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CONVERSION OF TOMATIDINE AND SOLASODINE INTO NEOTIGOGENIN[1] AND DIOSGENIN AND INTO A COMMON CONSTITUENT, 5α-22,25-EPOXYFUROSTAN-3β-OL

Sir:

The deamination of N-nitrosotomatidine[1,2] with 30% aqueous acetic acid in ethanol leads to a mixture from which neotigogenin[3] (8–10%), m.p. 197–199°, $[\alpha]_D$ −78.6° (chf),[4] (*Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.91; H, 10.53) was isolated. It was identical in all respects [derivative (acetate), m.p., mixture m.p., infrared spectrum[5]] with an authentic specimen. The major product is the isomeric 5α-22,25-epoxyfurostan-3β-ol, Ia, m.p. 178–180°, $[\alpha]_D$ −58° (chf); (*Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.85; H, 11.00). Acetylation of Ia leads to the acetate, Ib, m.p. 198–201°, (*Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.70; H, 9.89).

(1) We are indebted to Dr. C. L. Ruiz of Drogaco Industria Quimica S.A. for the exceedingly generous gift of tomatine.

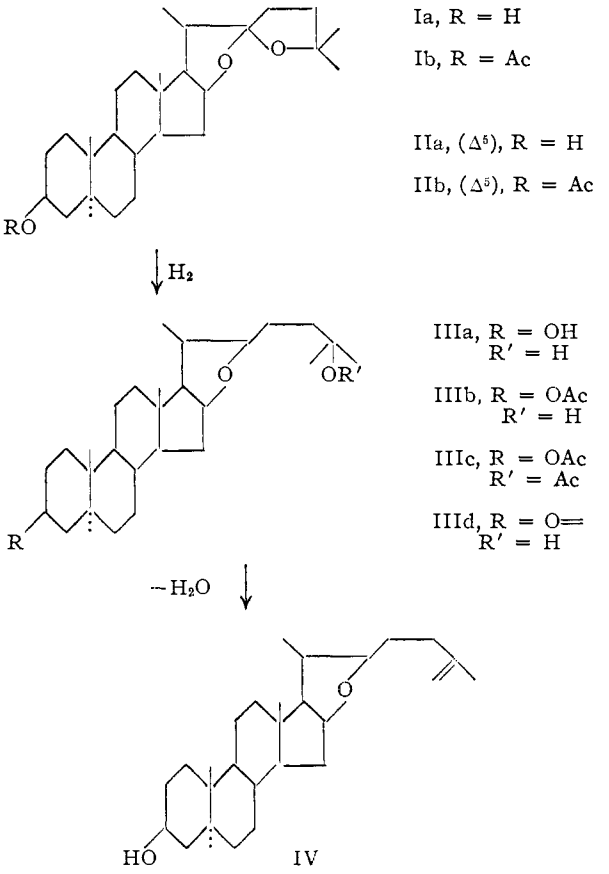
(2) Y. Sato, A. Katz and E. Mosettig, THIS JOURNAL, **74**, 538 (1952). The tomatidine for its preparation was purified through its hydrochloride by thoroughly washing the hydrochloride with dry ether and chromatographing the liberated aglycone. Dr. K. Schreiber of Forschungstelle für Kartoffel Käfer-Bekämpfung, Mühlhausen, Germany, has informed us that he has isolated a small amount of neotigogenin from crude tomatidine.

(3) All melting points are uncorrected and were taken on the Kofler block.

(4) Microanalyses are from the Institutes' service analytical laboratory under the direction of Dr. W. C. Alford.

(5) Infrared spectra were determined on a Perkin–Elmer double beam spectrophotometer by Mr. H. K. Miller of this laboratory.

In an analogous manner diosgenin (8–10%)[6] (m.p. 204–206°, $[\alpha]$ −125.4° (chf) *Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.52; H, 10.24) was obtained from N-nitrososolasodine.[7] Its properties were in agreement with an authentic sample of diosgenin. The major component in this mixture is 22,25-epoxyfurost-5-en-3β-ol[8] (IIa), m.p. 242–246°, $[\alpha]_D$ −110.6° (chf); (*Anal.* Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.28; H, 10.25). The acetate (IIb), m.p. 192–195°, (*Anal.* Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 75.91; H, 9.54), was prepared in the usual manner.



The mild catalytic reduction (platinum–acetic acid–methanol) of IIa yields the dihydro derivative, m.p. 178–180°, whose properties as well as those of its acetate (m.p. infrared spectrum, X-ray powder diagram[8]) were identical with Ia and its acetate obtained from tomatidine. Assuming an identical mechanism for the deamination process, this conversion of IIa into Ia strongly supports the view that the same spatial arrangement exists at C-22 in tomatidine and solasodine.

The structures of Ia as well as IIa were established by the following series of reactions:

(6) L. H. Briggs and T. O'Shea, *J. Chem. Soc.*, 1654 (1952). In an addendum to the above article, Briggs and O'Shea announced the isolation of diosgenin from N-nitrososolasodine in small amounts. Subsequently (THIS JOURNAL, **75**, 6067 (1953)) we isolated small amounts of diosgenin from crude solasodine.

(7) Solasodine was purified in the same manner as tomatidine.

(8) We are indebted to Mr. F. A. Hildebrand, National Geological Survey, Washington, D. C., for this determination.