

[CONTRIBUTION FROM THE DEPARTMENT OF COLLOID CHEMISTRY, D. J. MCCARTHY FOUNDATION, AND DEPARTMENT OF PHYSICS, TEMPLE UNIVERSITY SCHOOL OF MEDICINE]

Studies on Melanins.¹ III. X-Ray Diffraction Patterns of Photosynthetic and Genuine Melanins

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Although ultra-spectrographical investigations² have made it possible to distinguish several groups of melanins, no clues are as yet available as to the structure of the melanin molecule. X-Ray diffraction patterns of melanins have been made in an attempt to fill this gap.

Method.—X-Rays are provided by a gas type, self-rectifying X-ray tube³ used with a copper target and operated at 35 kv. peak at an average current of 15 m. a. Aluminum foil windows (0.013 mm.) and lead diaphragm 1 mm. in diameter and 47 mm. effective length are used. With this arrangement, a fairly monochromatic beam of X-rays of average wave length of 1.54 Å. is secured. The finely ground specimen is pressed into a hole drilled in a microscope slide.⁴ The specimen is at a distance of 88 mm. from the focal spot and of 37.7 mm. from the film (Eastman X-ray); the rigid construction of the cameras and of their attachment to the tube assures a correct alignment. The necessary time of exposure was found to be one hour. The table of Katz⁵ (corrected for our cameras) was used for the conversion of the diffraction rings into the plane distances in Ångström units.

Material.—The preparation and some properties of the three so-called photosynthetic melanins, *i. e.*, melanin-like products derived from phenylalanine, tryptophan, and tyrosine through the influence of ultraviolet light have been described before.⁶ The chemical as well as the ultraspectrographical properties of the samples of human tumor melanins as well as of two samples of sepia and a sample of mouse melanin have been given in a former paper.² All the preparations have been kept in powder form at room temperature.

Experimental Results.—All melanins show an X-ray diffraction pattern consisting of either one or two diffuse rings. The diameter of these rings as well as the corresponding planar separations are summarized in Tables I and II. Table I contains besides comparable data for phenylalanine, tryptophan and tyrosines, which were used as sources of photosynthetic melanins.

Conclusions

1. The three photosynthetic melanins show a similar diffraction pattern which is very different

(1) Read before the meeting of the American Chemical Society at Baltimore, Md., April 6, 1939.

(2) M. Spiegel-Adolf, *Fundamenta Radiologica*, **5**, 36 (1939).

(3) R. W. G. Wyckoff, *Rev. Sci. Instruments*, **7**, 35 (1936).

(4) W. T. Astbury and R. Lomax, *J. Chem. Soc.*, 896 (1935).

(5) J. R. Katz, "Die Roentgenspektrographie, etc.," *Handbuch der biol. Arbeitsmeth.* (Abderhalden) Abt. II., *Physikal. Meth.*, Teil 3, H. 6, p. 222, 1934.

(6) M. Spiegel-Adolf, *Biochem. J.*, **31**, 1303 (1937).

TABLE I^a

	Diameter, mm. ^b	Plan. Sep., Å.
Phenylalanine melanin	24 (6)	6.89
Tryptophan melanin	30 (5)	4.10
Tyrosine melanin	30 (4)	4.10
<i>dl</i> -β-Phenylalanine	16, 24, 30, 35, 38, 41, 53	
1-Tryptophan	6.5, 13, 21, 25, 32 (1.5),* 47, 55, 64	
1-Tyrosine	13, 18, 21, 25, 28, 36,* 41, 45, 50,* 54, 57, 62, 69, 76, 84	

^a The three photosynthetic melanins show besides evidence on an inner ring less than 9 mm. in diameter.

^b The figures in parentheses indicate the width of the ring, * indicates a band instead of a sharp line.

TABLE II^a

Melanins	Outer ring		Inner ring		Log E Å. = 3000
	Mm.	Å.	Mm.	Å.	
Sepia gen.	32 (8)	3.86	12	9.8	2.62
Sepia digest.	31	3.98	11	10.6	2.42
Hum. tumor F ₂	30	4.10	11	10.6	2.38
Hum. tumor F ₁	28	4.35	12	9.8	2.34
Hum. tumor P	28	4.35	11	9.8	2.30
Hum. tumor F ₃	27	4.5	11	9.8	..
Mouse melanoma	26 (4)	4.66	11	9.8	1.94

^a Melanins P and F₁ are chemically identical; F₃ shows only slight differences; F₂ larger differences.

from the elaborate sharp line pattern of their mother substances. Only tryptophan contains one diffuse ring similar to the one of tryptophan and tyrosine melanin. These findings confirm former observations of Spiegel-Adolf² according to which only irradiated tyrosine, but not phenylalanine, solutions gave a positive Hopkins-Cole reaction.

2. The diffraction pattern of the tumor melanins and of sepia although of greater intensity are similar to the ones of the photosynthetic melanins. There exist certain differences in the diameters of the various genuine melanins. These differences seem to run parallel to differences in chemical and optical properties of the genuine melanins.

3. The planar separations of the genuine melanins vary between 9.8–10.6 Å. and 3.86–4.66 Å. These values are close to the ones found by Astbury and his co-workers in proteins. (All our melanins were free from any protein contamina-

tion.) The interpretation of these results ought therefore to follow lines similar to the interpretation of the protein diffraction pattern. Either melanins must be considered as "anisotropic amorphous solid substances" according to Freundlich⁷ or as non-amorphous crystalline substances according to Astbury.⁴

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(7) H. Freundlich, "Kapillarchemie," Akad. Verlagsgesellschaft Leipzig, 1922, p. 999.

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Summary

X-Ray diffraction patterns of photosynthetic melanins as well as of the corresponding amino acids and of various tumor melanins and sepia are described. Slight differences observed in the diffraction patterns of the genuine melanins (from 9.8–10.6 Å. and from 3.86–4.66 Å.) run parallel with observed differences in the optical absorption power of the same substances.

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Stereochemistry of Biphenyls. XLVI.¹ 2-Substituted Biphenyls

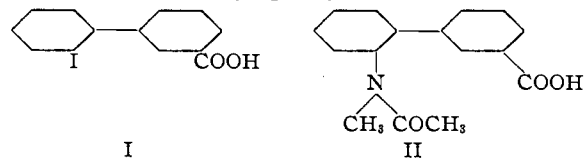
BY ROGER ADAMS AND T. L. CAIRNS²

The prediction of restricted rotation in biphenyls by means of calculated interferences based on atomic radii has proved very helpful. In 2,2'-disubstituted biphenyls, however, the actual stability of the optically active forms is less than would be anticipated from the stability of closely related 2,2',6- and 2,2',6,6'-tri- and tetra-substituted compounds. On these grounds, biphenyls monosubstituted in the 2-position should show even greater instability than atomic radii calculations would indicate. The published results of Meisenheimer³ and Lesslie and Turner⁴ confirm this. These latter investigators found it impossible to resolve the 3'-bromobiphenyl-2-tetramethylammonium iodide but obtained the corresponding tetramethylarsonium iodide as a mutarotating compound. In the same paper they mention that 2-iodo-3'-carboxybiphenyl was prepared and could not be resolved. However, this latter compound and its method of preparation were not described. The fact that 2,2'-diiodo-4,4'-dicarboxybiphenyl has been separated into two optically active forms⁵ indicates beyond a doubt that an iodine atom is sufficiently large to interfere with a hydrogen and consequently that,

unless some mobility greater than that found in 2,2'-disubstituted biphenyls is present in monosubstituted compounds, 2-iodo-3'-carboxybiphenyl should be resolvable.

The syntheses of biphenyls with a substituent in the 2-position and, in addition, a salt-forming group are difficult. 2-Amino-3'-carboxybiphenyl offers an attractive starting point for the preparation of such biphenyls since the amino group should be converted readily into or replaced by groups of large radii without difficulty. The details have now been determined by which this compound can be made from the condensation of *o*-bromonitrobenzene and ethyl *m*-iodobenzoate followed by reduction of the nitro group. This synthesis, which appears simple, required much experimentation before a satisfactory procedure was found.

In this communication the preparation of 2-iodo-3'-carboxybiphenyl (I) and N-acetyl-N-methyl-2-amino-3'-carboxybiphenyl (II) from 2-amino-3'-carboxybiphenyl is described. The



(1) For previous paper see Adams and Joyce, *THIS JOURNAL*, **60**, 1491 (1938).

(2) Solvay Process Company Fellow, 1938–1939. An abstract of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in chemistry.

(3) Meisenheimer and Beitswenger, *Ber.*, **65B**, 32 (1932).

(4) Lesslie and Turner, *J. Chem. Soc.*, 1588 (1933).

(5) Searle and Adams, *THIS JOURNAL*, **55**, 1649 (1933).

work of Lesslie and Turner was confirmed in that the iodo compound could not be resolved.

In view of the success of Mills and Kelham⁶ in

(6) Mills and Kelham, *J. Chem. Soc.*, 274 (1937).