$LiAlH_4$ wurde mit wasserhaltigem Äther zersetzt, die Reaktionsmischung durch Umschütteln mit 10%iger Natronlauge von ausgeschiedenem Aluminiumhydroxyd befreit, die ätherische Lösung mit Kaliumcarbonat getrocknet und eingeengt. Die hierbei ausgeschiedenen Kristalle wurden abgesaugt und aus Äther umkristallisiert. Feine Nadeln vom Schmp. 122～123°. Die Ausbeute: 3.5～3.6 g.

Drehung: 0.104 g. Substanz wurden in 95%igem Alkohol zu 5 ccm. gelöst. l=1 dm. α= 0.33°. $[\alpha]_D^{14}$:+15.8°

$C_{27}H_{38}O_3N_2$—Ber.: C, 73.97; H, 8.44; N, 6.39. Gef.: C, 73.58; H, 8.29; N, 6.71.

Aktive Wasserstoffbestimmung nach Zerewitinoff: 9.593 mg. Probe in Anisol entwickelten 1.054 ccm. Methan bei 0° und 760 mm.

$C_{27}H_{35}O_3N_2(H)_3$—Ber.: H, 0.68. Gef.: H, 0.50.

Zusammenfassung

N-Benzoylhydrocarbostyril wird beim Erwärmen mit verd. Salzsäure, Natronlauge bzw. mit alkoholischer Chlorwasserstoff-Lösung in 2-Benzoylaminohydrozimmtsäure bzw. ihren Äthylester übergeführt. Diese Reaktion wurde zur Ringaufspaltung vom Chinolin-Kern in Dihydrochinin übergetragen. 2′-Oxydihydrochinin wird durch Erhitzen von 2′-Chlordihydrochinin mit 20%iger Schwefelsäure mit besserer Ausbeute erhalten. Das letztere gibt bei der Durckhydrierung mit Raney-Nickel zwei stereoisomere 2′-Oxohexahydrochinin vom Schmp. 167～168° und vom Schmp. 125～135°. Das Mono- und Dibenzoat des ersteren wurden hergestellt. Der Lactam-Ring dieses Dibenzoates wird durch Erhitzen in alkoholischer Chlorwasserstoff-Lösung aufgespalten und das entsprechende Benzoylaminoäthylester erhalten. Bei der Reduktion des letzteren mit $LiAlH_4$ werden seine Ester- und Benzoylamino-Gruppen reduziert unter gleichzeitiger Verseifung der Benzoyloxy-Gruppe und der entsprechende Benzylaminoglykol erhalten.

(Eingegangen am 20. April, 1953)

---

39. Shoji Shibata and Shinsaku Natori: Metabolic Products of Fungi. II*. Metabolic Products of *Aspergillus amstelodami* (Mangin) Thom et Church.

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo***)

Metabolic products of *Aspergillus glaucus* group have been studied fully by Raistrick[1], and Quilico[2] and their co-workers, obtaining flavoglaucin (I), auroglaucin (II), erythroglaucin (III), parietin (IV), parietin anthranol A and B (V and VI), and echinulin (VII). The chemical structures of these products except echinulin have been well established.

Recently, the present authors were interested in the pigment formation of a strain of

* Part I: S. Shibata, S. Natori: J. Pharm. Soc. Japan, 71, 1167 (1951).

** Motofuji-cho, Bunkyo-ku, Tokyo (柴田承二, 名取信策).

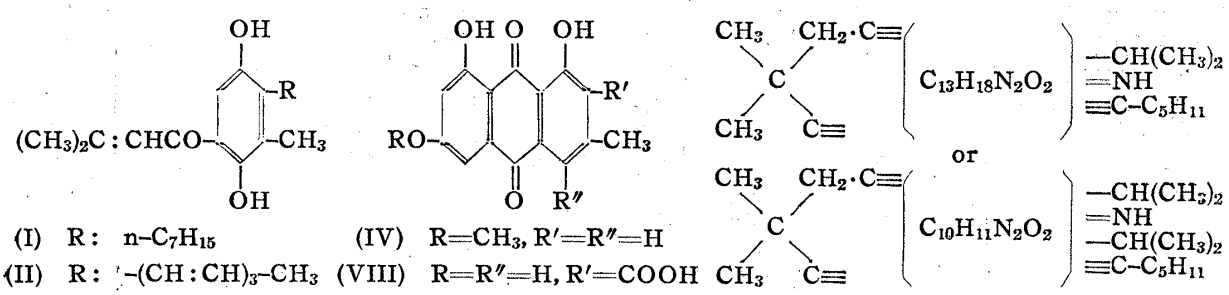
1) B. S. Gould, H. Raistrick: Biochem. J., 28, 1640 (1934); H. Raistrick, R. Robinson, A. R. Todd: J. Chem. Soc., 1937, 80; J. H. Cruickshank, H. Raistrick, R. Robinson: *Ibid.*, 1938, 2056; J. N. Ashley, H. Raistrick, T. Richards: Biochem. J., 33, 1291 (1939); J. H. Cruickshank, R. Robinson: J. Chem. Soc., 1938, 2064; W. K. Anslow, H. Raistrick: Biochem. J., 34, 1124 (1940).

2) A. Quilico, L. Panizzi: Ber., 76, 348 (1943) (C. A., 37, 5979); A. Quilico, L. Panizzi, V. Rosnati: Gazz. chim. ital., 78, 111 (1948) (C. A., 42, 6833); A. Quilico, L. Panizzi, V. Rosnati: Proc. XIth Intern. Congr. Pure & Applied Chem. (London), 2, 257 (1947) (C. A., 45, 7072); A. Quilico, L. Panizzi, E. Mugnaini: Nature, 164, 26 (1949); A. Quilico, L. Panizzi, E. Magnaini: Gazz. chim. ital., 79, 89 (1949) (C. A., 43., 7913); A. Quilico, C. Cardani, L. Panizzi: *Ibid.*, 80, 325 (1950) (C. A., 45, 3819); L. Panizzi, R. A. Nicolaus, A. Quilico: *Ibid.*, 80, 610 (1950); M. Simonetta, C. Cardani: *Ibid.*, 80, 750 (1950); A. Quilico, C. Cardani: Atti. accad. nazl. Lincei, 9, 220 (1950) (C. A., 45, 3909).

Aspergillus glaucus group isolated from plum juice*, which was identified as *Aspergillus amstelodami* (Mangin) Thom et Church**. During the preliminary testing it was shown that the fungus produced some pigments other than that recorded by the previous workers.

The mycelium of *Aspergillus amstelodami* cultivated in peptone-glucose media was extracted and the products were separated by the course as shown in Fig. 1. As had been reported by the earlier workers, flavoglaucin (I), auroglaucin (II), parietin (IV), and echinulin (VII) were isolated, and in addition, two crystalline pigments were found in the ethereal extract: An orange red compound of m.p. 309~312° (decomp.) (VIII), from the bicarbonate-soluble portion, and a ruby red compound of m.p. 244~246° (IX) from the carbonate-soluble portion. Under a certain cultural condition, N-containing yellow crystalline pigment of m.p. 255~257° (X) was obtained. By the examination of these compounds it became clear that the compound (VIII) was identical with endocrocin (2-methyl-4,5,7-trihydroxyanthraquinone-3-carboxylic acid), which had been isolated from a lichen, *Nephromopsis endocrocea* Y. Asashina[3], and the compound (IX) with catenarin (1,4,5,7-tetrahydroxy-2-methylanthraquinone), a fungal pigment first isolated from *Helminthosporium gramineum* Rabenhorst[4]. Due to its poor yield, a nitrogen-containing compound (X) could not be examined fully.

Occurrence of endocrocin in the products of *Aspergillus amstelodami* offers an additional example of a metabolic product produced by both lichen and fungi.



(I) R: n-C_7H_{15}

(II) R: -$(CH:CH)_3$-CH_3

(IV) R=CH_3, R'=R''=H

(VIII) R=R''=H, R'=COOH

(IX) R=R'=H, R''=OH

Hydrogenated product of (VII)

The authors wish to thank Prof. A. Quilico of Politecnico di Milano, and to Prof. H. Raistrick, London School of Hygiene and Tropical Medicine, for their kind supply of the authentic specimens of echinulin and catenarin, respectively. Thanks are also expressed to Mr. H. Kamata, Lecturer of Applied Chemistry of this University, and his collaborators for their kind cooperation in the measurment of the infrared spectra. Analyses were made by the members of the analytical laboratories of the Institute for Infectious Diseases and this Institute, to whom the authors are indebted.

Experimental

(The m.ps. given are uncorrected.)

Cultural conditions—The preliminary examination showed that the following medium was most satisfactory for the production of the pigments: Peptone 10 g., glucose 100 g., and NaCl 10 g., made to 1 L. with water (pH 5.4).

The sterilized media were inoculated from malt-agar slope cultures of *Aspergillus amstelodami* (Mangin) Thom et Church and incubated at 25° for 20~40 days. After 4 to 10 days' incubation, the mycelium grew over the surface of the media and the production of yellow pigment was observed, which turned to yellowish brown, then reddish brown. The color of the media also turned yellowish brown with a yellowish green fluorescence. The pH of the media after 30 days' incubation was 3.0. Yield of the mycelium harvested was 11~21 g. per 1 L. of media.

* The authors are indebted to Mr. I. Sasaki of Tsumura Laboratory for his kind supply of the strain of the fungus.

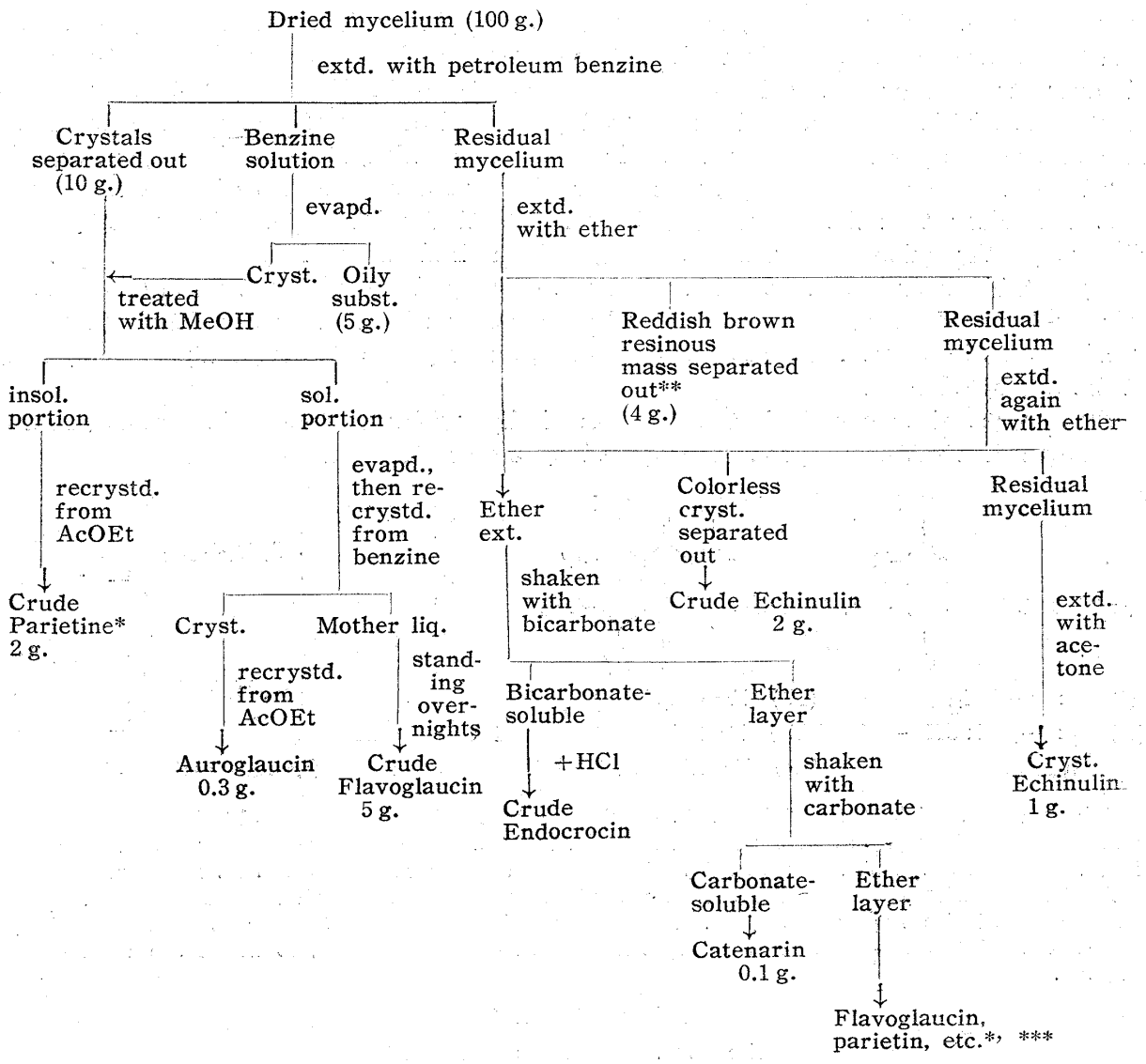
** The determination of the strain was performed by Dr. Y. Kobayashi of the Science Museum, Tokyo, to whom the authors' thanks are due.

3) Y. Asashina, F. Fuzikawa: Ber., 68, 1558 (1935).

4) J. Charles, H. Raistrick, R. Robinson, A. R. Todd: Biochem. J., 27, 499 (1933); H. Raistrick, R. Robinson, A. R. Todd: *Ibid.*, 28, 559 (1934); W. K. Anslow, H. Raistrick: *Ibid.*, 34, 1124 (1940).

Extraction and isolation of the metabolic products—The dried mycelium was extracted successively with petroleum benzine, ether, and acetone, and each extract was treated as shown in Fig. 1.

Fig. 1 Separation of the Metabolic Products of *Aspergillus amstelodami*



* Erythroglaucin was not detected in the fraction by paper chromatography.
** This fraction mainly consisted of parietin, but three spots (Rf 0.95, 0.35, and 0.10) appeared in the paper chromatogram of this fraction (Developing solvent: methanol-saturated benzine[5]), indicating that parietin was contaminated with an anthraquinone and an anthranol.
*** Some colorless crystals are present in this fraction.

Parietin: Recrystallization from ethyl acetate and sublimation under reduced pressure gave orange red needles of m.p. 204.5~205°. Mixed melting point measurement with authentic sample showed no depression. From the mother liquor separated from parietin, colorless needles which melted at 242~248° were obtained. Although further purification of the crystals was impossible due to the shortage of the product, it was assumed to be parietin-anthranol A from its specific reaction with SeO_2 and conc. H_2SO_4 giving black precipitates and the Rf-value of the paper chromatogram of its oxidation product in comparison with that of parietin[5].

Auroglaucin: Recrystallized from ethyl acetate and then from methanol giving orange red

5) S. Shibata, M. Takido, O. Tanaka: J. Am. Chem. Soc., 72, 2789 (1950).

needles, m.p. 152~153°. *Anal.* Calcd. for $C_{19}H_{22}O_3$: C, 76.48; H, 7.44. Found: C, 76.13; H, 7.38.

Flavoglaucin: Repeated crystallization from petroleum ether gave lemon yellow needles, m.p. 102~104°, which reacted with *o*-phenylenediamine to give yellow leaflets, m.p. 153~155°. *Anal.* Calcd. for $C_{25}H_{34}O_2N_2$: N, 7.10. Found; N, 7.21.

Echinulin: Crude echinulin obtained as shown in Fig. 1, was recrystallized from glacial acetic acid or alcohol to give colorless needles of m.p. 242~243°, which was proved to be identical with echinulin by a mixed fusion with the authentic specimen. *Anal.* Calcd. for $C_{28}H_{39}O_2N_3$: C, 74.83; H, 8.69; N, 9.35. Found: C, 74.54, 74.86; H, 8.25, 8.43; N, 9.48, 9.41.

Endocrocin—Attempt was made to purify the bicarbonate-soluble portion of the ethereal extracts by recrystallization and absorption chromatography, but gave unsatisfactory results.

The purification was made through acetylation. The acetate, m.p. 214~218°, was hydrolyzed and the product was recrystallized from 50% pyridine to give yellow long needles melting at 116~118°, which resolidified at 140~150°, then remelted at 308~310° (decomp.). Yield: 0.1~0.2 g. from 1 L. culture. *Anal.* Calcd. for $C_{16}H_{10}O_7 \cdot C_6H_5N$: N, 3.45. Found: N, 3.32. Further purification was carried out by sublimation in vacuo (240~260°, 0.008~0.02 mm. Hg) or by recrystallization from glacial acetic acid to form orange red prisms, m.p. 309~312° (decomp.). *Anal.* Calcd. for $C_{16}H_{10}O_7$: C, 61.18; H, 3.21. Found: C, 61.04, 61.24, 61.41; H, 3.39, 3.33, 3.37.

Since the mixed melting point determination of the product with the authentic specimen of endocrocin gave an obscure result due to its decomposition at melting point, the identity was confirmed by the Rf-value of paper chromatogram[5], and absorption bands of ultraviolet and infrared spectra (Figs. 2 and 3).

Acetate: Yellow needles, m.p. 216~219°, which gave no depression of m.p. by a mixed fusion with endocrocin triacetate, m.p. 213~216°. *Anal.* Calcd. for $C_{22}H_{16}O_{10}$: C, 60.00; H, 3.66. Found: C, 59.77; H, 3.64.

Methyl ether methyl ester: Yellow needles, m.p. 228~229°, which gave no melting point depression by mixed fusion with endocrocin trimethyl ether methyl ester, m.p. 226~228°. *Anal.* Calcd. for $C_{20}H_{18}O_7$: C, 64.86; H, 4.89; CH_3O, 33.52. Found: C, 64.57; H, 4.94; CH_3O, 33.18.

Methyl ether: Orange yellow needles, m.p. 229~232° (decomp.). *Anal.* Calcd. for $C_{19}H_{16}O_7$: C, 64.05; H, 4.56; CH_3O, 26.13. Found: C, 63.96; H, 4.79; CH_3O, 25.74.

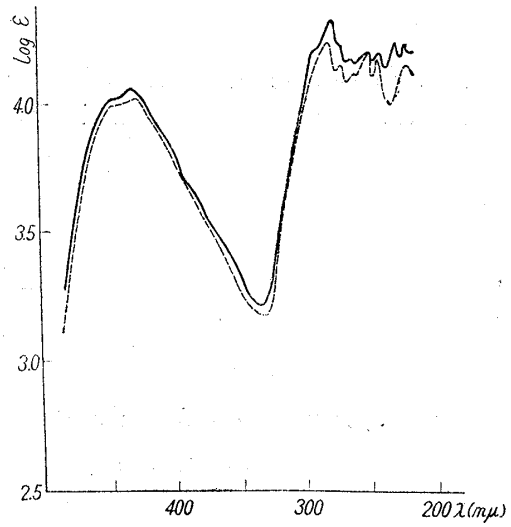


Fig. 2 Ultraviolet Absorption Spectra of Endocrocin and Emodin.
—— Endocrocin
- - - Emodin
1/10000 mol. alcoholic solution

Catenarin—The sodium carbonate-soluble fraction of the ethereal extract consisted of crimson powder, paper chromatography of which showed four spots when methanol-saturated benzine was employed as the developing solvent[5]: Rf, 0.90~0.95 (orange)*, 0.35~0.42 (violet, fluorescence), 0.05~0.10 (orange), and 0.0 (no coloration). The main fraction giving violet coloration with magnesium acetate was isolated as follows: The chloroform solution of the mixture was chromatographed through the column of a magnesium oxide-Celite mixture (1:2), and eluted by chloroform, then with methanol. Each fraction was examined by paper chromatography, and the fractions which gave a simple main spot were combined and the solvent was removed. The red colored residue was acetylated with acetic anhydride and a drop of conc. H_2SO_4 to yield lemon yellow acetate, m.p. 232~233° (from ethyl acetate). Admixture with catenarin acetate showed no melting point depression. *Anal.* Calcd. for $C_{23}H_{18}O_{10}$: C, 60.79; H, 3.99. Found: C, 61.04; H, 3.96.

On hydrolysis the acetate gave ruby red plates, m.p. 244~246°, when purified by recrystallization from ethanol and sublimation in vacuo, which was proved to be identical with catenarin by a mixed fusion.

Nitrogen-containing substance of m.p. 255~257°—In the preliminary examination the fungal mycelium grown on Kinoshita's medium[6] yielded an N-containing compound. The crystalline substance that separated out during the extraction of the mycelium with ether was treated with methanol, when a yellow crystalline substance was obtained after a long standing at a room temperature. The compound recrystallized from methanol melting at 255~257° was sparingly soluble in caustic alkali but gave gradually faint violet red coloration; it gave red color with conc. H_2SO_4, and no coloration with ferric chloride. It gave a positive reaction of the presence of N in

* Color developed by magnesium acetate reagent.

6) K. Kinoshita: Misc. Repts. Research Inst. Nat. Resources (Japan), **17~18**, 77 (1950).

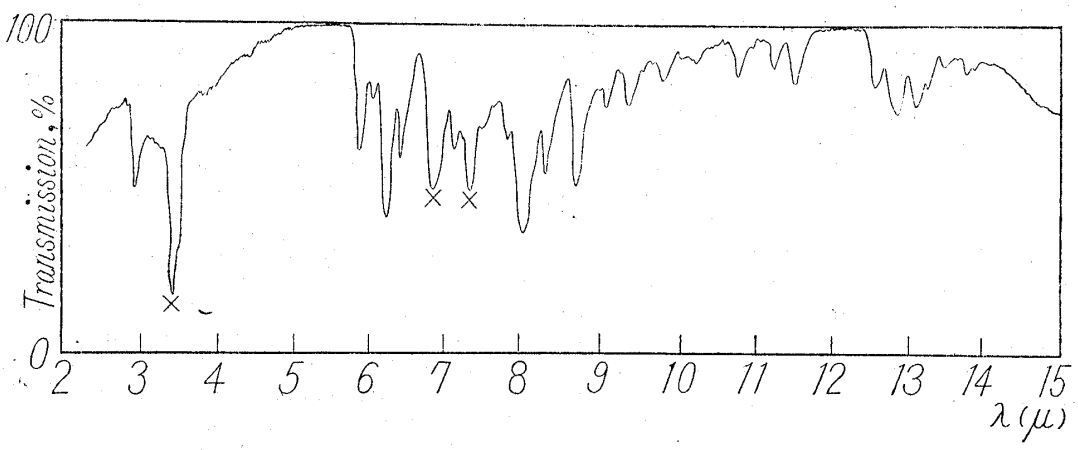


Fig. 3 Infrared Absorption Spectrum of Endocrocin (in Nujol)
× Nujol absorption

its molecule, but negative for S and halogen. *Anal.* Found: C, 71.04; H, 6.02; N, 13.30. Due to its poor yield, further study was not made.

Summary

The metabolic products of *Aspergillus amstelodami* (Mangin) Thom et Church were investigated, and endocrocin and catenarin were newly isolated.

(Received April 20, 1953)

---

40. Ken'ichi Takeda and Wataru Nagata: Components of the Root of *Lindera strychinifolia* Vill. V.[1] Azulenes isolated from Linderene by Zinc-Dust Distillation.

(*Research Laboratory, Shionogi & Co., Ltd.**)

In the previous paper of this series[2], one of the authors (Takeda) reported the isolation of linderazulene as purple crystals by the zinc-dust distillation of linderene[3]. This azulene can also be obtained by heating linderene with selenium or palladium carbon.

In the present series of experiments, the present authors reconfirmed these facts and also isolated a new azulene, aside from linderazulene, as blue oil. The new azulene, $C_{14}H_{16}$, confirmed as its picrate and a trinitrobenzene complex, did not coincide with any of the azulenes reported in literature and was designated as lindazulene.

I. Isolation of azulenes

a) From linderene: The mixture of azulenes obtained by the zinc-dust distillation of linderene was treated in the usual manner and purified through chromatography, the column being developed carefully with a mixture of petroleum ether and ether (100 : 3).

The two principal components adsorb at F_2 and F_4 portion of alumina column (Fig. 1). Lindazulene is isolated from the F_2 portion and linderazulene from F_4, showing that the

* Imafuku, Amagasaki, Hyogo-ken (武田健一, 永田 亘).

1) Part IV: J. Pharm. Soc. Japan, 64, 154 (1944). 2) Part III: *Ibid.*, 59, 504 (1939).
3) For examples of obtaining azulenes by zinc-dust distillation, cf. J. Am. Chem. Soc., 53, 3507 (1941).