246. *Lichens and Fungi. Part I. Polyporic Acid in* Stictae.

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The lichens *Sticta coronata* and *S. colensoi* yield principally polyporic acid, calycin, pulvinic lactone, and neutral substances. The former species also contains pulvinic acid, two red quinonoid pigments, a phenol, and other substances.

Polyporic acid is the main pigment of the fungus *Peniophora filamentosa*.

AMONG the lichens, various species of *Sticta* (fam. *Stictaceae*) are notable for their yellow or orange colours. Nine members of the genus have been investigated by Zopf (*Annalen*, 1879, **297**, 271; 1899, **306**, 282; 1901, **317**, 110; 1904, **338**, 35), Hesse (*J. pr. Chem.*, 1900, **62**, 321), and Asano and Kamedo (*Ber.*, 1935, **68**, 1568), who found them to contain the orange-yellow pigment, stictaurin. No depsides appear to have been isolated from these lichens, although two species, *S.* (*Stictina*) *gilva* Thbg and *S. glaucolurida* Nyl. (Thies, in Klein's "Handbuch der Pflanzenanalyse," Vol. III, i, p. 434), yielded in addition to the pigment colourless neutral substances, stictinin and stictalbin respectively. From *S.*

coronata Muell. Arg. [*S. orygmaea* Ach. (?Hook.)], Zopf also obtained a brown, sparingly soluble pigment, "orygmaeic acid," m. p. 310°, which gave purple salts with alkalis. This substance has apparently not been reported since and nothing is known of its constitution, although Zopf suggested that it might be an anthraquinone.

During a recent investigation of New Zealand lichens, the pigments and other substances have been extracted from a number of *Stictae* including two related species, *S. coronata* Muell. Arg. and *S. colensoi* Bab. which yielded orygmaeic acid.

Sticta coronata, although yellow in texture, gives deep red solutions on extraction with organic solvents, being thus readily distinguished from *S. colensoi* Bab., *filix* Hoffm., *flavicans* Hook., *impressa* Hook., and *glaucolurida* Nyl. which are also yellow but give yellow solutions with ether or acetone. The dried plant gave a considerable yield of acetone-soluble material, from which were isolated orygmaeic acid (1·7%), calycin (3·2%), pulvinic lactone (0·7%), pulvinic acid (0·35%), compounds A (0·07%), B (0·02%), C (0·02%), and D (0·08%), and other substances (*ca.* 3%). Of these, compounds A and D are believed to be new, while pulvinic acid has apparently not been found previously in Nature, although the lactone and the methyl ester (vulpinic acid) have been isolated from many lichens.

"Orygmaeic acid" appeared to be identical with polyporic acid (2 : 5-dihydroxy-3 : 6-diphenylbenzoquinone) obtained from the fungus *Polyporus nidulans* Pers. by Stahlschmidt (*Annalen*, 1877, **187**, 177; cf. Kögl, *Annalen*, 1926, **447**, 78), and this was confirmed by direct comparison with an authentic specimen synthesized by the method of Shildneck and Adams (*J. Amer. Chem. Soc.*, 1931, **53**, 2373).

Compound A, a red pigment, is easily oxidised by air and has perhaps not been obtained completely pure. Analyses are best for a molecular formula $C_{14}H_{10}O_7$. At least three oxygen atoms are present as phenolic hydroxyl groups and two as a quinone group. The colour reactions of the substance are similar to those of anthragallol, but no recognisable product was obtained by zinc dust distillation and the pigment appears not to be an anthraquinone. It forms a neutral yellow acetate which is rapidly decomposed by sodium hydroxide to a mixture of colourless water-soluble products.

Compound B is also a hydroxy-quinone pigment, similar in general properties to compound A, but more stable to oxidation.

Compound D has properties typical of a group of about twenty neutral unreactive substances which have been isolated from lichens (see Thies in Klein's "Handbuch der Pflanzenanalyse," Vol. III, i). It is a colourless, highly crystalline substance, slightly soluble in most solvents, and optically active. It contains no methoxyl groups, shows no carbonyl or hydroxyl group reactions, and is unaffected by acid or alkali. Despite this lack of reactivity, the infra-red absorption spectrum has a strong carbonyl band at 1742 cm.$^{-1}$. There is no absorption corresponding to hydroxyl groups. Although there is a weak absorption band in the ultra-violet below 220 mμ, the substance appears to be saturated and the infra-red spectrum shows no ethylenic absorption. Compound D may be a triterpenoid, but owing to our inability to prepare any derivatives of it, we have not yet been able to define accurately its molecular formula.

Sticta colensoi Bab. [*S. urvillea* Del. var. *colensoi* Nyl., *S. endochrysa* Fl. Ant., *S. durvillea* (Del) of Zahlbr.] gives about 5% of acetone extractives, comprising mainly polyporic acid and calycin with a small amount of pulvinic lactone. The red pigments of the previous species were absent; small amounts of two substances similar to compound D were obtained from the neutral fraction. Neutral substances identical with, or similar to, compound D have been isolated, sometimes in considerable yield, from several of the other *Sticta* species which have been examined.

In a search for polyporic acid from fungal sources, a considerable number of the available species belonging to the related genera *Polyporus* and *Fomes* were extracted. However, although fair yields of red and brown quinone-type pigments were obtained from a few species, none contained polyporic acid. The investigation was extended to fungi of the family *Thelephoraceae*, and from one of these, *Peniophora filamentosa* (B. & C.) Burt, polyporic acid was obtained in 5% yield. Preliminary tests of several other *Peniophora* and *Corticium* species (not yet identified) indicated the presence of pigments similar to polyporic acid, while descriptions in the botanical literature (*e.g.*, Burt, *Annals Missouri*

Bot. Gard., 1925, **12**, 213) suggest that pigments of this type may be widely distributed in these genera. It is perhaps worthy of note, however, that polyporic acid cannot be distinguished by simple colour tests from thelephoric acid, a phenanthraquinone pigment occurring in several members of the allied genus *Thelephora* (Zopf, *Bot. Zeit.*, 1889, **47**, 69) and in a lichen (Asahina and Shibata, *Ber.*, 1939, **72**, 153).

EXPERIMENTAL

Extraction of Sticta coronata.—The lichen, collected from trees at Port Pegasus, New Zealand, in January, was dried (450 g.) and extracted with warm acetone till the extracts were nearly colourless. The total extract was concentrated to about 150 c.c. and the crude crystallised pigments were filtered off, further small crops of calycin and polyporic acid being obtained by concentration of the filtrate. The crystalline material (25 g.) was extracted with the minimum amount of boiling acetone, leaving purple cubes of polyporic acid (7·2 g.). Stictaurin crystallised from this acetone extract as golden-yellow plates, m. p. 212°, followed by mixtures of calycin and pulvinic lactone in varying proportions and with m. p.s between 210° and 235°. These mixtures were separated by extracting a benzene solution with sodium hydrogen carbonate to remove traces of polyporic acid, then with very dilute sodium hydroxide solution which dissolved the calycin as the red sodium salt, leaving the pulvinic lactone in the neutral layer. Alternatively, the calycin–pulvinic lactone mixture was boiled with ethanol, which converted the lactone into ethyl pulvinate, leaving the calycin unchanged. The resulting mixture was readily separated by crystallisation from alcohol. The approximate yields of calycin and pulvinic lactone were 14·5 and 3 g. respectively.

The dark-red syrup (13 g.) obtained on evaporation of the first acetone mother-liquors was taken up in ether and extracted in turn with (*a*) aqueous sodium hydrogen carbonate and (*b*) aqueous sodium hydroxide, leaving (*c*) a straw-coloured neutral ethereal solution.

(*a*) The bicarbonate extract was acidified and extracted several times with ether. The extracted solution was still red, and on storage for several months slowly deposited a brown amorphous powder (250 mg.), charring above 370°, which has not been characterised. After removal of the ether, the syrupy residue was left in chloroform; a dark-brown semicrystalline material (i) slowly separated. This was removed and slow evaporation of the filtrate gave a crop of reddish-yellow powder (ii), followed by plates and small needles of *compound A* (240 mg.), and leaving a red syrup (iii).

Material (i) was extracted several times with warm acetone, leaving a residue of crude polyporic acid. Impure pigment B separated when the acetone extracts were cooled, and recrystallised from hot xylene as chocolate-brown plates (100 mg.). The xylene mother-liquors on further crystallisation yielded small amounts of polyporic acid, pigment B (5 mg.), and calycin (2 mg.).

The amorphous product (ii) (1·76 g.) was extracted (Soxhlet) with light petroleum to remove a little yellow oil, then a portion of it (1·15 g.) was boiled with ethanol, cooled, filtered to remove tarry material, diluted to 80% with water, and left for several days. The crystalline product which appeared gave orange-red needles of compound A (50 mg.) on recrystallization from 90% ethanol. The ethanolic mother-liquors were evaporated to a brown residue which was extracted with boiling light petroleum to remove a small amount of compound A. The insoluble residue (600 mg.) consisted of pulvinic acid. Alternatively, the mixture of compound A and pulvinic acid could be resolved by treatment with acetic anhydride and acetic acid at the b. p. The pulvinic acid was converted by this treatment into pulvinic lactone, and compound A formed a water-soluble yellow resin from which no useful material was obtained.

The syrup (iii), when kept in benzene, gave a crop of red amorphous material (220 mg.) and then was recovered as a pale reddish glass (3·0 g.). The latter gave a complex series of bands on alumina or magnesia, indicating the probable presence of five coloured substances, but the mixture could not be satisfactorily separated by chromatography. On sublimation up to 220°/0·02 mm. the only volatile products were about 30% of pulvinic lactone, m. p. 222° (ethyl ester, m. p. 127°), and traces of calycin, m. p. 245°, a white crystalline acid, m. p. 115°, a red liquid acid, and a liquid phenolic decomposition product. The pulvinic lactone is presumably derived from pulvinic acid in the crude material.

(*b*) The red-brown sodium hydroxide extract rapidly became purple, then black, on exposure to the air. On acidification it gave a black resin (3·0 g.) which was now partly soluble (1·1 g.) in sodium hydrogen carbonate solution. The remaining phenolic fraction crystallised from acetone as a white microcrystalline solid, m. p. 312° (90 mg.) (compound C). Evaporation of the acetone mother-liquor gave a red-brown powder which could not be crystallized from any solvent. On treatment with acetic anhydride–acetic acid it formed a black insoluble neutral tar. Small amounts of calycin and acetylcalycin were obtained from the tar.

(*c*) The syrup obtained from the neutral ethereal solution gave white crystals of *compound D* (390 mg.) on slow evaporation of ethereal or methanolic solutions. The compound was then readily purified by crystallisation from hot alcohol. The bulk of the neutral fraction appeared as an amorphous, nearly colourless powder (*ca.* 4 g.) which has not been investigated.

Polyporic Acid.—The pigment was obtained as purple cubes from hot acetone, brown scales from xylene, or red needles from pyridine (losing pyridine on exposure to the air and falling to a brown powder), all of m. p. 315° (bath preheated to 310°). The m. p. determined in the ordinary way is 305—307° (decomp.). The spectrum in pyridine has maxima at 494 and 388 mμ (log ε 2·115 and 3·35), and the red solution becomes blue on dilution with water ($\lambda_{max.}$ 530 mμ). Zinc dust distillation gave a very poor yield of a non-picrate-forming hydrocarbon, m. p. 205°, presumably terphenyl. The diacetate was obtained in quantitative yield by refluxing the pigment with acetic anhydride and acetic acid for 30 minutes. It crystallised in yellow needles from benzene, or orange prisms from nitromethane, m. p. 212° (Found: C, 69·6; H, 4·1. Calc. for $C_{22}H_{16}O_6$: C, 70·2; H, 4·3%).

Synthetic polyporic acid was prepared by the route: benzoquinone, 2 : 5-diphenylbenzoquinone (25% yield), 2 : 5-diphenylquinol (80%), 2 : 5-dibromo-3 : 6-diphenylbenzoquinone (90%), polyporic acid (100%). Purification of the intermediate products was unnecessary. The spectrum of the synthetic sample was identical with those of the natural products, and the m. p.s and mixed m. p.s of the four samples of polyporic acid and their acetates were identical.

Calycin and Pulvinic Lactone.—Calycin was obtained as orange-red needles, m. p. 248°, from ethanol. Unlike pulvinic lactone, it is stable to prolonged boiling with alcohols. The acetate, prepared by refluxing of calycin in a large excess of acetic anhydride for 5 hours and cooling, separated as yellow needles (from ethanol or acetic acid), m. p. 179°. Pulvinic lactone crystallised in yellow needles, m. p. 227°, from benzene. The lactone, when heated under reflux in ethanol for 1 hour, gave pale yellow plates of the ethyl ester, m. p. 128°. The m. p. of ethyl pulvinate is only slightly depressed by admixture with calycin, both substances melting fairly distinctly close to their correct m. p.s.

Compound A.—The *compound* was obtained as small red needles (from chloroform), m. p. 227—228° [Found: C, 58·0; H, 4·1%; *M* (micro-electrometric titration), 294. $C_{14}H_{10}O_7$ requires C, 57·95; H, 3·45%; *M*, 290]. Crystallisation from 95% ethanol gave orange-red needles [Equiv., by microhydrogenation (one quinone group), 295±8], and from 10% ethanol–water, deep-red silky needles, m. p. 230° (decomp.) (Found: C, 54·2; H, 3·9. $C_{14}H_{10}O_7,H_2O$ requires C, 54·5; H, 3·9. The sample was dried *in vacuo* at room temperature). The compound is very soluble in ether and acetone and fairly soluble in other solvents, slightly so in light petroleum or water. It forms intense purple lakes on alumina and magnesia, from which it is not readily recovered in a crystalline state. The solution in acetic acid is orange-red (impure specimens may give a slight greenish fluorescence); in sodium hydrogen carbonate solution, it is dark greenish-purple (absorption bands in blue and yellow regions), in dilute ammonia green (becoming red, then brown), in sodium hydroxide blue-purple (rapidly bleaching in air), and in concentrated sulphuric acid yellow-brown (unchanged by addition of boric acid). It gives a deep greenish-brown colour with ferric chloride. The pK_a values are 5·8 (green), 7·5 (blue), and *ca.* 10 (purple). Its absorption max. (mμ) and log ε in various solvents are tabulated.

EtOH (abs.) ...	—	502	474	—	337	—	—
Dioxan	—	—	—	—	337	274	244
EtOH (90%) ...	—	520	470	—	340	272	241
	—	3·64	3·696	—	3·828	4·240	4·192
4N-NaOH	675—650	580	500	402	314	261·5	—
	3·46	3·60	3·57	3·42	4·04	4·20	—
H_2SO_4	650—630	518	483·5	—	288·5	—	—
	(3·0)	3·975	3·734	—	4·065	—	—
H_2SO_4–B_2O_3 ...	675—630	522	487	462 (infl.)	290·5	—	—
	(3·2)	4·314	4·094	3·867	4·466	—	—

Distillation of the pigment with zinc dust gave a very small yield of a white volatile substance, m. p. 20—25°, which did not form a picrate.

Acetate. Attempted acetylation with acetic anhydride or acetyl chloride in the presence of sulphuric acid, sodium acetate, or pyridine led to yellow water-soluble tars. Compound A

(34 mg.) was kept in cold acetic anhydride (0·5 c.c.) with 1 drop of 60% perchloric acid for 1 hour, then poured into cold water. The resulting orange *triacetate* (25 mg.) crystallised from ethanol–acetic acid as yellow needles, m. p. 193—194° (decomp.) [Found : C, 57·2; H, 3·4%; *M* (microhydrogenation), *ca.* 380. $C_{20}H_{16}O_{10}$ requires C, 57·6; H, 3·85%; *M*, 416. The molecular weight from microhydrogenation is probably low, owing to partial hydrogenolysis, as the recovery of the acetate from the leuco-compound was not quantitative]. The spectrum of the acetate in 90% ethanol showed maxima at 360, 300, 263, and 230 mμ.

A substance which may be the acetate of the leuco-compound was prepared by treatment of the compound A with zinc dust in acetic anhydride and acetic acid. It separated from ethanol–acetic acid as pale fawn-coloured needles, m. p. 105—107° (decomp.), but has not been obtained pure.

Compound B.—The chocolate-coloured plates (from xylene) melted indefinitely with decomposition at 335—340°. *B* was obtained as red needles, m. p. 330—340°, from ethanol. The m. p. was not improved by repeated recrystallisation from xylene or toluene, and it could not be satisfactorily crystallised from other solvents. It gives blood-red solutions in oxygenated solvents, brown ones in aromatic hydrocarbons, and is insoluble in light petroleum or water. The compound formed a deep violet band on alumina or magnesia, which could be eluted only with glacial acetic acid. This did not effect any resolution and the properties of the substance were unchanged on recovery. The solution in acetic acid is bright red, in aqueous sodium hydrogen carbonate red, in sodium hydroxide purple (red in very dilute solution and stable to aerial oxidation), in sulphuric acid violet, and in sulphuric–boric acid purple. The spectra of the last two solutions (to be reported later) are markedly different. Compound B gives a dark green colour with ferric chloride.

Pulvinic Acid.—The compound was obtained as large brown crystals from ethanol, falling to a yellow powder, m. p. 215°, on exposure to the air. Volhard (*Annalen*, 1894, **282**, 1) gives m. p. 215—216° for synthetic pulvinic acid (Found, in a sample dried at 100° : C, 70·0; H, 4·4. Calc. for $C_{18}H_{12}O_5$: C, 70·1; H, 3·9%). Pulvinic acid gives yellow solutions in alkalis and orange, fading to yellow, ones in concentrated sulphuric acid. It gives no colour with ferric chloride and does not reduce silver nitrate. Refluxing the compound with excess of acetic anhydride for 1 hour gave a quantitative yield of pulvinic lactone, m. p. 220° (Found : C, 73·9; H, 3·6. Calc. for $C_{18}H_{10}O_4$: C, 74·4; H, 3·5%). The m. p. could not be raised by crystallisation, but a mixture with pulvinic lactone, m. p. 226°, had m. p. 223°, and a sample was converted into ethyl pulvinate, m. p. 127°.

Compound D.—The *compound* was obtained as glistening white plates, m. p. 259°, from methanol or ethanol [Found, in a sample dried at 100°/0·01 mm. : C, 72·4; H, 9·4%; *M* (Rast), 525, 535. $C_{32}H_{50}O_6$ requires C, 72·45; H, 9·4%; *M*, 530. $C_{34}H_{48}O_6$ requires C, 72·0; H, 9·3%; *M*, 516]; it had $[\alpha]_D^{18}$ +54° (*c*, 0·02 in dioxan). The ultra-violet spectrum shows an apparent maximum at 216 mμ (ε *ca.* 220). The absorption in dioxan at 220 mμ is 190; at 230 mμ, ε is 100; and at 270—280 mμ, ε is *ca.* 9. Compound D is moderately soluble in dioxan and acetone, slightly in most other solvents except water. It was recovered unchanged after being heated with acetic anhydride in acetic acid or pyridine for 4 hours, or with boiling alcoholic or 50% aqueous potassium hydroxide at 150°. It did not react with semicarbazide or dinitrophenylhydrazine. It was not hydrogenated in presence of Adams's catalyst in glacial acetic acid, did not absorb bromine, and gave no colour with tetranitromethane. The compound was insoluble in cold concentrated sulphuric acid but dissolved on warming to an orange solution with bright green fluorescence. The Liebermann–Burchard reaction gave an immediate violet-red colour, fading to yellow after 3 hours. The infra-red spectrum (in Nujol) shows absorption (in cm.$^{-1}$) at 1742 (vs), 1296 (m), 1258 (s), 1240 (vs), 1190 (m), 1156 (w), 1105 (w), 1081 (w), measured on a Perkin–Elmer Model 21 instrument.

Extraction of S. colensoi (with G. A. NICHOLLS, Canterbury University College).—The plant (collected in the Waimakariri Valley, N.Z.) was extracted essentially as for *S. coronata*, giving polyporic acid (1·2%), calycin (3·2%), pulvinic lactone (0·2%), and colourless neutral substances (0·3%).

Extraction of Peniophora filamentosa.—This species is not uncommon in the north temperate zone, but has apparently not been reported previously from New Zealand. The whole dried plant (10 g.) (collected from a dead branch in March) was extracted with boiling ether for 8 hours. Polyporic acid (0·53 g.) crystallised from the hot solution and was filtered off. The filtrate on concentration gave brown and purple plates (20 mg.), then a yellow oil from which a trace of white wax, m. p. *ca.* 40°, slowly separated. The second crop of pigments was refluxed with acetic anhydride–acetic acid for 10 minutes; when the mixture cooled, grey-brown scales,

m. p. 360° (decomp.) (12 mg.), crystallised, leaving the contaminating polyporic acid in solution as the diacetyl derivative. This grey substance crystallises from pyridine as a violet-brown salt but was not otherwise characterised.

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