1133. *The Pigments of Stick Lac. Part I. Isolation and Preliminary Examination*

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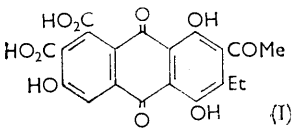
Laccaic acid contains at least four components. Methods have been devised for separating these and for obtaining pure the major component, laccaic acid A_1. This compound, of probable molecular composition $C_{26}H_{21}NO_{12}$, is a quinone-carboxylic acid containing four acetylatable hydroxyl groups (three arranged as in purpurin), one *C*-Me group, and a non-basic nitrogen atom which can be estimated by the van Slyke method. The chromophore of laccaic acid A_1 is probably that of an anthraquinone modified by further conjugation.

The insect *Laccifer lacca* Kerr, found on the twigs of certain trees native to India and South-East Asia, produces a resin known as stick lac.[1] Extraction of stick lac with dilute sodium carbonate solution removes pigments and leaves behind shellac. Treatment of the extract with chalk or alum precipitates lac-dye which, until the advent of synthetic dyes, was of considerable commercial importance.

From lac-dye Schmidt [2] isolated material named laccaic acid. The isolation was effected by leaching the powdered dye with dilute acid. The crude pigment from the acid extract was purified by decomposing its lead salt with hydrogen sulphide and recrystallisation from water. Schmidt made preliminary studies of the chemistry of laccaic acid, but a more detailed investigation was carried out by Dimroth and Goldschmidt,[3] who isolated laccaic acid by extracting powdered stick-lac with water. Concentration of the extract and acidification with hydrochloric acid precipitated crude laccaic acid as a red solid which was recrystallised from formic acid. Chemical studies, summarised by Thomson,[4] led Dimroth and Goldschmidt to a partial formulation of laccaic acid as a polysubstituted anthraquinone. This was elaborated by Mayer [5] to the expression (I). Tschirch and Ludy [6] obtained support for the presence of the anthraquinone nucleus from zinc dust distillation studies. Evidence for the presence of nitrogen in laccaic acid was obtained by Kamath and Potnis [7] by analysis of the crude calcium salt of laccaic acid, but the nature of their preparation could not generate confidence in the result. Recently Venkataraman and his co-workers found laccaic to be a mixture of pigments containing 1—2% of nitrogen present as $-NH_2$.[8] A second pigment isolated by Tschirch and Ludy [6] from stick lac, and called erythrolaccin was recently formulated as 1,3,5,6-tetrahydroxy-8-methylanthraquinone.[9]



(I)

It is the purpose of this Paper to report work carried out on laccaic acid, isolated from stick lac essentially by the method of Dimroth and Goldschmidt,[3] in which the major component of laccaic acid has been obtained pure.

Laccaic acid represented about 1% by weight of stick lac, as was also found by Dimroth

[1] "Shellac," Angelo Brothers Ltd., Calcutta, 1956.
[2] R. E. Schmidt, *Ber.*, 1887, **20**, 1285.
[3] O. Dimroth and S. Goldschmidt, *Annalen*, 1913, **399**, 62.
[4] "Naturally Occurring Quinones," by R. H. Thomson, Butterworths, London, 1957.
[5] "The Chemistry of Natural Coloring Matters," by F. Mayer, trans. by A. H. Cook, Reinhold Publishing Corporation, New York, 1943.
[6] A. Tschirch and F. Ludy, *Helv. Chim. Acta*, 1923, **6**, 994.
[7] N. R. Kamath and S. P. Potnis, *Paint India*, 1953, **3**, No. 1, 107.
[8] N. S. Bhide, R. Srinivasan, and K. Venkataraman, *Abstracts of Papers*, I.U.P.A.C. Symposium (Japan), "The Chemistry of Natural Products," (1964), p. 205.
[9] P. Yates, A. C. Mackay, L. M. Pande, and M. Amin, *Chem. and Ind.*, 1964, 1991; N. S. Bhide, A. V. Rama Rao, and K. Venkataraman, *Tetrahedron Letters*, 1965, 33.

and Goldschmidt,[3] though the yield was considerably influenced by the conditions used in the extraction process. Prolonged storage of powdered stick lac before extraction with water, or storage of the extract reduced the yield.

Early paper chromatographic studies of laccaic acid, using n-butanol–acetic acid–water (4 : 1 : 5) (System I), revealed two well-defined spots. The substances responsible for the spots were named laccaic acid A and B, respectively. Later work revealed a more complex situation; use of the system ethyl acetate–chloroform–10% aqueous trichloroacetic acid (1 : 1 : 2) (System II) uncovered three components of laccaic acid. These were a stationary component (laccaic acid B), and two components, laccaic acid A_1 and A_2, which together formed the single spot attributed to " laccaic acid A " when System I was used.

System II had obvious disadvantages for use in preparative column chromatography, and a search was made for other systems capable of separating laccaic acids A_1 and A_2 from laccaic acid B on a cellulose column. The most useful found was prepared from ethyl acetate, acetic acid, and water (4 : 1 : 5) (System III). This system could be used to separate the components on paper. The aqueous phase was used to spray the paper chromatogram before development with the organic phase. Laccaic acids A_1 and A_2 appeared together as a single spot whilst laccaic acid B remained at the origin. An artifact produced in the treatment of the crude pigment with methanol moved at the solvent front.

The use of System III in cellulose column chromatography gave the artifact mentioned above as the first coloured eluate from the column. Fractional collection of the main band showed that at its head there was a mixture containing laccaic acids A_1 and A_2 and a small quantity of artifact, in which laccaic acid A_2 predominated. From the major portion of the main band, consisting predominantly of laccaic acid A_1 with some A_2, the former could be obtained pure by recrystallisation from 85% formic acid, or preferably, methanol. The use of a methanol-containing solvent to remove the last band from the column split the band into a new component (laccaic acid C) and laccaic acid B. The latter could be purified by recrystallisation. This chromatographic separation has been applied to 10 g. batches of crude laccaic acid. A typical run is summarised in Table 1. The method uncovered at least four components of crude laccaic acid; of these laccaic acid A_1, laccaic acid A_2, and laccaic acid B, represented approximately 50, 30, and 5%, respectively, of the mixture.

Paper chromatography of material obtained by the procedure of Dimroth and Goldschmit showed that they probably used material consisting predominantly of laccaic acid A_1 contaminated with variable amounts of laccaic acids A_2 and B.

Chemical studies have been confined to laccaic acid A_1 which was obtained as bright red crystals that sintered at 230° and gradually charred at higher temperatures. The pigment was slightly soluble in polar solvents. It dissolved in organic bases and dilute alkaline solutions including sodium hydrogen carbonate, in which it gave a purple colour and liberated carbon dioxide. Solutions in concentrated sulphuric acid were magenta coloured and showed no fluorescence.

Analysis of laccaic acid A_1 from separate preparations gave carbon 57·6—57·9, hydrogen 3·7—3·9, and nitrogen 2·3—2·5%. The figures for carbon and hydrogen agree closely with those reported by Schmidt [2] and by Dimroth [3] but neither of these workers recorded the presence of nitrogen. Potentiometric titrations of laccaic acid A_1 in dimethyl sulphoxide revealed three strongly acidic hydrogen atoms and gave a molecular weight of 546. Titration in aqueous solution gave an equivalent weight of 185, corresponding to the molecular weight 555. The analytical and molecular-weight data lead to a probable molecular formula of $C_{26}H_{21}NO_{12}$ for laccaic acid A_1 which is supported by data for its derivatives. Kuhn–Roth and Zerewitinoff determinations indicated that one *C*-methyl group and from seven to eight active hydrogen atoms are present; *O*- and *N*-alkyl groups were absent.

Acetylation of laccaic acid A_1 gave a yellow crystalline solid, which analysis suggested

to be a tetra-acetate associated with one molecule of acetic acid. A tendency for one molecular equivalent of acid to become strongly associated with the pigment was also noticed when samples of laccaic acid were recrystallised from formic acid.

Laccaic acid A_1 showed quinonoid properties but absorbed 1·8—1·9 molecular equivalents of hydrogen on reduction over a platinum catalyst in methanol. The yellow solution obtained rapidly turned red on exposure to the air and analysis suggested the product to be a dihydrolaccaic acid A_1. The molecular weight was established by potentiometric titration, osmometry, and microhydrogenation which gave values of 552, 540, and 539, respectively. Catalytic hydrogenation of dihydrolaccaic acid A_1 followed by ærial oxidation gave back starting material. Acetylation of dihydrolaccaic acid A_1 under conditions similar to those used for the parent compound gave an amorphous yellow compound which may be a tetra-acetate, but its ultraviolet spectrum suggests that other changes may have occurred.

Alkaline sodium dithionite solution converted laccaic acid A_1 into an orange crystalline product, xantholaccaic acid A_1. Potentiometric titrations showed that this also contained three acidic hydrogen atoms and gave molecular weights of 533 and 540. Xantholaccaic acid A_1 rapidly absorbed one molecular equivalent of hydrogen when reduced in methanol over platinum. The reduction could be stopped at this stage and the product reconverted into xantholaccaic acid A_1 by exposing the solution to air. On prolonged reduction a very slow further uptake of hydrogen was noted but the total hydrogen absorbed did not approach two molecular equivalents. The analytical figures for several preparations of xantholaccaic acid A_1 were consistent with a molecular formula of $C_{26}H_{23}NO_{12}$, apparently produced from laccaic acid A_1 by the reductive removal of one oxygen atom and the addition of a water molecule. Xantholaccaic acid A_1 contained one *C*-methyl group and 8—9 active hydrogen atoms. On acetylation the compound gave an amorphous triacetate. Both this and the acetate from dihydrolaccaic acid A_1 were shown to be homogeneous by paper chromatography and consistent analyses.

The ready reducibility of laccaic acid A_1, and the quinonoid properties of dihydrolaccaic acid A_1 and xantholaccaic acid A_1, clearly indicate a quinonoid system in the parent pigment. With methanolic magnesium acetate and with boroacetic anhydride in acetic anhydride the compound gave deep purple and purple-red colorations, respectively. These results suggest that laccaic acid A_1 contains at least one *peri*-hydroxyl group at each carbonyl.[10,11] The infrared spectra of laccaic acid A_1 and its acetate fully support these findings. The pigment shows bands in the infrared carbonyl stretching region at 1715, 1677, and 1620 cm.$^{-1}$. The 1715 cm.$^{-1}$ peak is attributed to a carboxylic acid group, while the peak at 1620 cm.$^{-1}$ is consistent with a 1,4-quinone in which both carbonyl groups are chelated by a total of two or three *peri*-hydroxyl groups.[12] We are not able at present to ascribe the 1677 cm.$^{-1}$ band to a functional group, but in view of the ultraviolet data believe that it may be due to a non-quinonoid carbonyl group. In laccaic acid A_1 acetate the carbonyl region showed bands at 1771 and 1672 cm.$^{-1}$, and inflections at 1734 and 1717 cm.$^{-1}$. The first two are attributable to phenolic acetate and unchelated 1,4-quinone groups, respectively.

Xantholaccaic acid A_1 gave a brown-orange colour with magnesium acetate. With boroacetic anhydride an orange colour was obtained. These results suggest a quinone with only one chelated carbonyl group. The infrared spectrum supported these conclusions. It showed bands at 1703, 1675, and 1623 cm.$^{-1}$ which we attribute to carboxylic acid, unchelated quinone carbonyl, and chelated quinone carbonyl, respectively. The removal of a *peri*-hydroxyl group by sodium dithionite is typical of a 1,2,4-trihydroxyanthraquinone system.[13] On this evidence we postulate a 1,2,4-trihydroxyl pattern in

[10] S. Shibata, M. Takito, and O. Tanaka, *J. Amer. Chem. Soc.*, 1950, **72**, 2789.
[11] O. Dimroth and T. Faust, *Ber.*, 1921, **54**, 3020.
[12] H. Bloom, L. H. Briggs, and B. Cleverley, *J.*, 1959, 178.
[13] E. de B. Barnett and J. W. Cook, *J.*, 1922, 1376.

an aromatic ring adjacent to the quinone ring in laccaic acid A_1. Venkataraman and his co-workers [8] also concluded that a purpurin system is present.

The band in the visible spectrum of laccaic acid A_1 (see Figure 1) has a maximum at 497 mμ and shows some fine structure. This position and feature are characteristic of quinones with two *peri*-hydroxyl groups in one adjacent ring although this has not always been recognised for anthraquinone systems. Briggs *et al.*[14] considered a maximum in the region 427·5—432·5 mμ to be characteristic of anthraquinones with two *peri*-hydroxyl groups as in 1,4-, 1,5-, or 1,8-dihydroxyanthraquinones, and this has been accepted by later authors.[15–17] With a slight extension of the higher limit the generalisation is true for anthraquinones with two *peri*-hydroxyl groups in different rings. However, when the two groups are in the same ring the absorption maximum is in the region which Birkinshaw and Gourlay consider typical for three *peri*-hydroxyl groups; [16] for example, 1,4-dihydroxyanthraquinone has $\lambda_{max.}$ 486 mμ in hexane [18] and close to 472 mμ in methanol [19] while

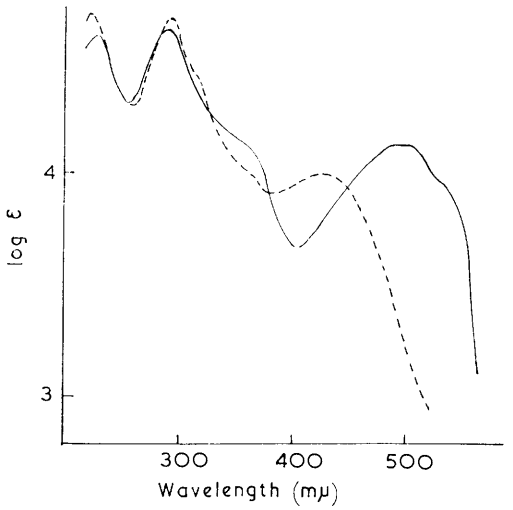


FIGURE 1. ——— Laccaic acid A_1 (MeOH). – – – – Xantholaccaic acid A_1 (MeOH)

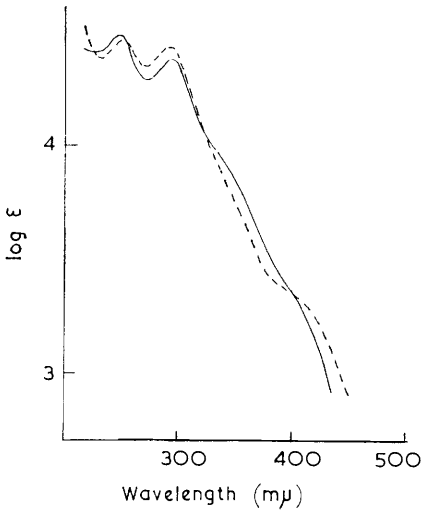


FIGURE 2. ——— Laccaic acid A_1 acetate (MeOH). – – – – Xantholaccaic acid A_1 acetate (MeOH)

purpurin has a visible maximum at 482 mμ in methanol.* The visible maximum in the spectrum of xantholaccaic acid A_1 is at 426 mμ and the band shows no fine structure (see Figure 1), properties which lend further support to the evidence cited above for the presence of one chelated carbonyl group in this compound. In the ultraviolet region the spectra of laccaic acid A_1 and xantholaccaic acid A_1 were almost identical (Figure 1). The positions of the peaks at 287 and 294 mμ are not compatible with a naphthazarin system.[20] Anthraquinone systems commonly show multiple bands between 260 and 300 mμ [15,16,19] but occasionally this region contains only one intense band.[15–17] In

* The ultraviolet absorption spectrum of purpurin in methanol shows $\lambda_{max.}$ 227, 257, and 482 mμ, log $\varepsilon_{max.}$ 4·21, 4·49, and 3·88; λ_{infl} 283, 464, 504, and 541 mμ; log ε_{infl} 3·99, 3·83, 3·76, and 3·21 (present work).

[14] L. H. Briggs, G. A. Nicholls, and R. M. L. Paterson, *J.*, 1952, 1718.
[15] B. H. Howard and H. Raistrick, *Biochem. J.*, 1955, **59**, 475; J. H. Birkinshaw, *ibid.*, p. 485.
[16] J. H. Birkinshaw and R. Gourlay, *Biochem. J.*, 1961, **80**, 387.
[17] E. Bullock, D. Kirkaldy, J. C. Roberts, and J. G. Underwood, *J.*, 1963, 829.
[18] R. A. Morton and W. T. Earlam, *J.*, 1941, 159; T. Ikeda, *J. Pharm. Soc. Japan*, 1956, **76**, 217.
[19] C. J. P. Spruit, *Rec. Trav. chim.*, 1949, **68**, 325.
[20] A. K. Macbeth, J. R. Price, and F. L. Winzor, *J.*, 1935, 325; J. W. H. Lugg, A. K. Macbeth, and F. L. Winzor, *J.*, 1937, 1597.

the latter cases, with one exception, the anthraquinone system contains at least two β-hydroxyl or β-alkoxyl groups and the intensity of the band exceeds log ε 4·3. The exception is 4,5,7-trimethoxy-2-methylanthraquinone [17] which has $\lambda_{max.}$ 277 mμ (log ε 4·41). The detailed spectrum of laccaic acid A_1 does not correspond to any of the published anthraquinone spectra. A principal ultraviolet maximum in naphthacenequinones [21] also occurs in the region 280—300 mμ, and the same is true for 1,2-benzanthraquinones.[22] It was hoped that more conclusive evidence for the chromophore would come from the ultraviolet spectra of laccaic acid A_1 acetate and xantholaccaic acid A_1 acetate since it has been accepted [23] that acetates of polyhydroxy-quinones have ultraviolet spectra which closely resemble those of the parent quinones. They showed, respectively, $\lambda_{max.}$ 251 and 295 mμ (log ε 4·49 and 4·39) and 254 and 294 mμ (log ε 4·48 and 4·44) (Figure 2). However, in neither case was there a well defined quinonoid band, but only slight shoulders at about 394 mμ. The remaining portions of the spectra are similar to those of 1,4,6-triacetoxynaphthacenequinone and 1,2-benzanthraquinone. In view of these features, and the fact that the position of the visible peak in xantholaccaic acid A_1 falls outside the ranges common to anthraquinones containing one α-hydroxyl, or two α-hydroxyl groups in different rings,[12] it seems likely that laccaic acid A_1 has not a simple anthraquinone chromophore, but one which is extended, possibly by a further aromatic ring which may be carbocyclic or heterocyclic.

The chromophore of dihydrolaccaic acid A_1 (see Experimental section) is very different from that of laccaic acid A_1. The addition of hydrogen must have been to the chromophore of laccaic acid A_1, and since xantholaccaic acid A_1 is not readily reduced in this way the 1,4-dihydroxy-system probably facilitates the reduction.

Titration of laccaic acid A_1 with perchloric acid in glacial acetic acid gave no evidence of the presence of a basic nitrogen atom. However, the nitrogen in laccaic acid A_1 and dihydrolaccaic acid A_1 could be estimated by the method of van Slyke. This fact, reported also by Venkataraman and his co-workers,[8] is presumably due to the generation of a primary amino-group under the conditions of the estimation. In contrast, the nitrogen in xantholaccaic acid A_1 could not be estimated by the van Slyke method.

Experimental

R_F values refer to the ascending solvent method, using Whatman No. 1 paper. Pigments were applied to chromatograph papers in methanol solution. Ultraviolet absorption spectra were measured in methanol, and infrared spectra in KBr discs.

Isolation of Crude Laccaic Acid (*Typical Run*).—Stick lac (2 kg.) was coarsely ground by hand and freed from twigs, and then powdered in a Raymond Laboratory Mill (International Combustion Ltd.) fitted with a coarse mesh sieve. Powdering was carried out slowly to avoid heating.

Freshly powdered material was stirred with water (20 l.) for 4 days, and the suspension was allowed to settle overnight. Decantation from the sludge, filtration through glass wool, and centrifuging (5 min. at 2500 r.p.m.) gave a clear wine-red solution. This was concentrated at water-pump pressure to 2 l. by means of a cyclone evaporator, the temperature being kept below 40°. The concentrate was freed by centrifuging from a small amount of purple solid, and then acidified with concentrated hydrochloric acid (400 ml.). The suspension was kept for 24 hr. and centrifuged. The precipitate was centrifuged with water until the solutions were no longer acid to Congo Red, and the solid was collected. Drying *in vacuo* over calcium chloride gave laccaic acid (13·1 g.) as a bright red solid. De-fatting with boiling chloroform removed 10—15% of the crude laccaic acid as wax.

Laccaic acid showed a major spot (laccaic acid A_1 + A_2) at R_F 0·41 and a minor one (laccaic acid B + C) at R_F 0·16 on paper chromatograms using System I. A paper sprayed with the aqueous layer and developed with the organic layer of System II showed laccaic acid A_1 (R_F 0·14), laccaic acid A_2 (R_F 0·08), and laccaic acid B + C (stationary). The red spots became

[21] H. Brockmann and W. Müller, *Chem. Ber.*, 1959, **92**, 1164.
[22] K. Alder and M. Schumacher, *Chem. Ber.*, 1956, **89**, 2485.
[23] W. D. Treadwell and G. Schwarzenbach, *Helv. Chim. Acta*, 1928, **11**, 386.

purple when treated with ammonia. Chromatographic examination of the liquors from which laccaic acid had been precipitated suggested that laccaic acid B was the major pigment remaining in solution.

Column Chromatography.—Whatman standard grade cellulose powder (600 g.) was spread out on a large photographic tray and sprayed with System III (aqueous phase) (120 ml.). The cellulose was immediately slurried in System III (organic phase) and used to prepare a column (153 × 4·8 cm.).

De-fatted laccaic acid (10 g.) was dissolved in methanol (1 l.) by Soxhlet extraction. Cellulose powder (70 g.) was added, and whilst the mixture was stirred vigorously (magnetic stirrer) the solvent was evaporated under reduced pressure at room temperature. The solid residue was finely powdered, thoroughly dried *in vacuo*, slurried in a small volume of System III (organic phase) and transferred to the prepared column.

Elution with System III (organic phase) was begun at a rate of 500 ml./hr. When all the main band had been eluted and the eluate was colourless, the eluant was changed to methanol–acetic acid–water (90 : 5 : 5) (System IV). Each fraction was evaporated at reduced pressure and room temperature under nitrogen, and the residues were dried to constant weight. The components of each fraction was ascertained by circular paper chromatography (Systems I and II). The results of a typical run are tabulated below:

TABLE 1

Fraction	Wt. (g.)	Volume of eluant (ml.)	Fraction of crude pigment (%)	Components	Eluant
1	0·40	250	3·9	Artifact	System II
2	3·70	400	36·0	Laccaic acids A_2, artifact, and trace of A_1	System III
3	5·03	2800	49·0	Laccaic acids A_1 and A_2 with trace of artifact	System III
4	—	3750	—	Tailings of laccaic acids A_1 and A_2	System III
5	0·40	750	3·9	Laccaic acid C	System IV
6	0·54	2500	5·2	Laccaic acid B	System IV

Purification of Material from Fraction No. (3 + 4).—The solid (5·5 g.) was dissolved in boiling methanol (300 ml. reflux) and the hot solution was filtered. The filtrate was concentrated to about 80 ml. and kept overnight. The product (2·4 g.) was redissolved in boiling methanol. Concentration to about 50 ml. gave crystals which were collected and washed with ether (5 × 10 ml.). Drying *in vacuo* gave laccaic acid A_1 (2·0 g.) as small, red, strongly dichroic platelets, which started to char at 230° (Found: C, 57·7, 57·9, 57·6; H, 3·8, 3·8, 3·9; N, 2·5, 2·4, 2·3; O, 36·05; NH_2 (van Slyke), 2·8, 2·85; *C*-Me, 2·4, 2·3; Active H, 1·2. Required for $C_{26}H_{21}NO_{12}$: C, 57·9; H, 3·9; N, 2·6; O, 35·6; NH_2, 3·0; *C*-Me, 2·8%). Titration of a solution of the pigment in dimethyl sulphoxide with 0·1N-tetra-n-butylammonium hydroxide (in methanol–benzene) revealed three dissociations corresponding to equivalent weights 600, 298, and 182. These correspond to molecular weights 600, 596, and 546. Titration in aqueous dimethyl sulphoxide (3% of dimethyl sulphoxide by volume) with 0·1N-sodium hydroxide solution showed one dissociation corresponding to Equiv. 185 (*M*, 555) (*M* of $C_{26}H_{21}NO_{12}$: 539·4).

Microhydrogenation was carried out in a Clauson–Kaas apparatus. Laccaic acid A_1 (19·80 mg.) was stirred with Adams catalyst (8·6 mg., previously reduced) in methanol (5 ml.). The red solution became orange and finally pale yellow. 1·502 ml. of hydrogen (corrected to N.T.P.) were absorbed in about 12 hr., the first half of this amount being absorbed rapidly [Calc. uptake (2 molecular equivs.) for $C_{26}H_{21}NO_{12}$: 1·644 ml.]. This uptake corresponds to a reduction equivalent of 295, and in other runs values from 292·5—298·5 were obtained.

The ultraviolet absorption spectrum showed $\lambda_{max.}$ 229, 287, 339 (infl.), 477 (infl.), 497, 528 (infl.) mμ; log ε 4·61, 4·64, 4·19, 4·08, 4·14, 3·97. The infrared spectrum (KBr disc) showed $\nu_{max.}$ 3385, 1715, 1677, 1620, 1567, 1500, 1437, 1399, 1370, 1284, 1222, 1097, 1049, 1005, 959, 870, 832, and 772 $cm.^{-1}$.

Recrystallisation of Fraction No. 6.—The solid (0·66 g.) was dissolved in 50% aqueous acetic acid (160 ml., reflux). After hot filtration the solution was concentrated to about 40 ml. The product was washed with 50% aqueous acetic acid (4 × 5 ml.) and ether (10 × 5 ml.). Laccaic acid B formed small dark red crystals, m. p. >360° (Found: 52·4, 52·3, 52·5; H, 3·5, 4·0, 4·0; N, 3·0, 2·65, 2·5; Active H, 0·9%).

Laccaic Acid A_1 Tetra-acetate.—Laccaic acid A_1 (497 mg.) was shaken with acetic anhydride (25 ml.) and concentrated sulphuric acid (10 drops). The red suspension quickly gave an orange solution which on standing (18 hr.) became yellow. The liquid was poured on ice (150 ml.) and the yellow solution was extracted continuously with ethyl acetate. The solvent was removed *in vacuo* from the dried (Na_2SO_4) extract, and the residue was freed from traces of solvent by storage in a desiccator over sodium hydroxide. The yellow froth was crystallised from acetic acid (3 ml.), giving the tetra-acetate as yellow prisms (540 mg.), m. p. 178° (Found: C, 56·3; H, 4·3; N, 1·5; acetyl, 27·5. $C_{34}H_{29}NO_{16},C_2H_4O_2$ requires C, 56·4; H, 4·3; N, 1·8; acetyl, 28·1%); $\lambda_{max.}$ 251, 295 mμ (log ε 4·49, 4·44); $\nu_{max.}$ 3413, 1771, 1672, 1573, 1433, 1370, 1189, 1113, 1097, 1009 cm.$^{-1}$.

Dihydrolaccaic Acid A_1.—Laccaic acid A_1 (280 mg.) was shaken with Adams catalyst (115 mg.), methanol (100 ml.), and hydrogen. Uptake was complete in 8 hr. The filtered solution was exposed to the atmosphere for 1 day whereupon it became dark red. Solvent was removed *in vacuo* and the residue was crystallised from water (110 ml.) containing conc. hydrochloric acid (11 ml.). *Dihydrolaccaic acid A_1* formed small, dark red rods (191 mg.), m. p. 205—210° (decomp.) (Found: C, 57·6; H, 4·0; N, 2·6; NH_2 (van Slyke), 2·85; Active H, 1·4. $C_{26}H_{23}NO_{12}$ requires C, 57·7; H, 4·3; N, 2·6; NH_2, 3·0%); $\lambda_{max.}$ 225, 275, 347, 447, 493 mμ (log ε 4·53, 4·52, 4·25, 3·80, 3·69); $\nu_{max.}$ 3356, 1724, 1667, 1611 (infl.), 1592, 1500, 1437, 1365, 1293, 1235, 1096, 1043, 953, 882, 835, 807 cm.$^{-1}$; *M* (vapour pressure osmometry in MeOH), 537, 540, 543, 546; (from hydrogenation equivalents, Adams catalyst in MeOH) 517, 518, 539; (from titration by 0·1N-sodium hydroxide) 552, 555 ($C_{26}H_{23}NO_{12}$ requires *M*, 541).

Acetylation of Dihydrolaccaic Acid A_1.—The acid (90 mg.) was shaken with acetic anhydride (5 ml.) and conc. sulphuric acid (2 drops) to give a red solution. On standing (15 hr.) this became greenish-yellow. It was poured into ice–water (50 ml.) and the resulting yellow solution was extracted continuously with ethyl acetate. Concentration *in vacuo* of the dried (Na_2SO_4) estract gave a yellow-brown glass. This could not be crystallised, but was obtained from acetic acid–ether as a mustard-yellow powder (65 mg.), m. p. 170° (decomp.) (Found: C, 57·8; H, 4·1; N, 2·4; acetyl, 22·9. $C_{34}H_{31}NO_{16}$ requires C, 57·5; H, 4·4; N, 2·0; acetyl (tetra-acetate), 24·3%); $\lambda_{max.}$ 261, 311, 359 (infl.), 459 mμ (log ε 4·52, 4·20, 3·98, 3·54); $\nu_{max.}$ 3399, 2933, 1769 (sh), 1673 (sh), 1599, 1423, 1365, 1269, 1187, 1072, 1024 cm.$^{-1}$.

Xantholaccaic Acid A_1.—Laccaic acid A_1 (259 mg.) in 5% sodium carbonate solution (20 ml.) was treated with a solution of sodium dithionite (761 mg.) in water (20 ml.). The original purple solution of laccaic acid was rapidly decolourised. After 4 hr. the yellow-brown solution was acidified (Congo Red) with 50% hydrochloric acid. The canary-yellow solution was warmed to expel sulphur dioxide, cooled, and extracted with carbon disulphide (2 × 20 ml.). Sodium ions were removed from the solution by passage over Zeo-Karb 225 (H^+ form) (30 × 2 cm.) and elution with water. The eluate (400 ml.) was extracted continuously with ethyl acetate (24 hr.) and the dried (Na_2SO_4) extract was concentrated *in vacuo* to give a yellow-orange solid (210 mg.). *Xantholaccaic acid A_1* was obtained from aqueous acetic acid (1 : 9) as small orange crystals (156 mg.), m. p. 210° (decomp.) [Found: C, 57·9; H, 4·0; N, 2·6; *C*-Me, 3·2, 3·4; Active H, 1·5; NH_2 (van Slyke), 0·0. $C_{26}H_{23}NO_{12}$ requires C, 57·7; H, 4·3; N, 2·6; *C*-Me, 2·8%]; *M* (hydrogenation), 534, 549, 576, 589; (titration with Bu_4NOH in dimethyl sulphoxide) (3 dissociations) 602, 554, 540 ($C_{26}H_{23}NO_{12}$ requires *M*, 541·5); $\lambda_{max.}$ 223, 294, 315 (infl.), 426 mμ (log ε 4·70, 4·68, 4·45, and 4·00); $\nu_{max.}$ 3370, 2909, 1703, 1675, 1623, 1576, 1423, 1365, 1313, 1274, 1250, 1231, 1165, 1139, 1099, 1038, 910, 889, 870, 823, 759 cm.$^{-1}$.

Xantholaccaic Acid A_1 Acetate.—The acid (130 mg.), acetic anhydride (5 ml.), and conc. sulphuric acid (2 drops) were shaken until solution was obtained and then kept for 18 hr. The yellow solution was poured into ice–water (40 ml.) and then continuously extracted with ethyl acetate. Evaporation of the dried (Na_2SO_4) extract *in vacuo* gave a yellow solid. The acetate was obtained from aqueous acetone as a mustard-yellow powder, m. p. 175° (decomp.) (Found: C, 57·5; H, 4·3; N, 1·8; acetyl, 19·5. $C_{32}H_{29}NO_{15}$ requires C, 57·6; H, 4·4; N, 2·1; acetyl (triacetate), 19·3%). $\lambda_{max.}$ 254, 294 mμ (log ε 4·48 and 4·44).

We thank May and Baker for leave and support granted to one of us (D. E. W.) and for some microanalyses, and to the D.S.I.R. for a maintenance grant (to R. B.).

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[*Received, April* 23*rd,* 1965.]