of the elements in a quotient are summed and the square root is extracted to yield the relative standard deviation of the quotient (8). When this is done with the 10% uncertainty mentioned previously, a relative random error of at least 10% is expected in the result. On this basis, only 1.4% of the original 11.4% remains unexplained. This percentage may very well be random error in AE for which no estimate is available.

During the rapidly rising portion of the curve, the sensing and recording of potentials is most difficult since the potential is changing very rapidly. Factors such as speed of recorder response, efficiency of stirring, and rate of titrant addition are all involved in this measurement. It is possible to obtain an electronic setup whereby the rate of titrant addition is slowed as a function of the rate of change of potential. This may improve the reproducibility of the slope. However, this feedback mechanism substantially increases the cost of the instrument. Commercial titrators offer this advantageous feature.

SUMMARY

1. A procedure for evaluating titration behavior of an automatic potentiometric titrator is reported.

2. The procedure consists of an instrument evaluation followed by an examination of titration curve properties. The range, end point potential, analytical results, and the slope of the rapidly rising portion of each curve are determined.

3. The maximum anticipated relative errors are calculated and compared with experimental results obtained from a relatively inexpensive titrator composed of commercially available units.

4. On the basis of satisfactory instrumental response and of reasonable titration curve errors, the titrator used here is found to be reliable for general laboratory use.

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Notes

Isolation of Aurantiacin from *Hydnellum caeruleum*

By M. L. MONTFORT*, V. E. TYLER, JR., and L. R. BRADY

Two procedures were employed for the isolation of aurantiacin from an ether extract of the basidiomycete *Hydnellum caeruleum*. Identification of the compound was based on its melting point, spectral properties (infrared, visible, and ultraviolet), and alkaline degradation to atromentin and benzoic acid.

POLYPORIC ACID (2,5-dihydroxy-3,6-diphenyl-1,4-benzoquinone) was shown by Burton and Cain to be the constituent responsible for the antitumor properties of the lichen *Sticta orygmaea* Ach. (*S. coronata* Müll. Arg.) (1). Subsequently, the antitumor activity of some related synthetic diaryldihydroxyquinones (2, 3) has been recorded. Recently, atromentin [2,5-dihydroxy-3,6-bis(4′-hydroxyphenyl)-1,4-benzoquinone] was identified as the anticoagulant principle in *Hydnellum diabolus* Banker (4, 5). Thus, compounds of this type have therapeutic potential, and their occurrence in nature has medicinal interest.

No diphenylbenzoquinone compound is known to occur in spermatophytes, but aurantiacin (atromentin-2,5-dibenzoate) (6), leucomelone (3′-hydroxyatromentin) (7), muscarufin [2,5-di(2′-carboxyphenyl) - 3 - hydroxy - 6 - (4′ - carboxy - 1,3 - buta-

Received April 11, 1966, from the Drug Plant Laboratory, College of Pharmacy, University of Washington, Seattle 98105.
Accepted for publication August 26, 1966.
Presented to the Pharmacognosy and Natural Products Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.
This investigation was supported in part by grant GM 07515-06 from the U. S. Public Health Service, Bethesda, Md.
* Winner of the 1966 Kilmer Prize.

dienyl)-1,4-benzoquinone] (8), thelephoric acid [2,3,8,9-tetrahydroxybenzobis(1,2-*b*, 4, 5-*b'*)-benzofuran-6,12-quinone] (9–14), and polyporic acid (15–21), in addition to atromentin (4, 22, 23), have been isolated from certain basidiomycetes belonging to the genera *Amanita, Cantharellus, Hydnellum, Hydnum, Lopharia, Paxillus, Polyporus,* and *Thelephora.* The *Hydnaceae* appear to be a particularly rich source of the diphenylbenzoquinone compounds with *Hydnellum, Hydnum,* and *Lopharia* species yielding atromentin, aurantiacin, polyporic acid, and thelephoric acid. *Hydnellum caeruleum* (Pers.) Karst. is known to lack anticoagulant properties (5), but the species has never been investigated chemically. When a quantity of this species became available, it was considered desirable to examine the fungus for diphenylbenzoquinone constituents.

EXPERIMENTAL AND RESULTS

Material and Extraction Procedure.—Carpophores of *H. caeruleum* were collected, cleaned, and dried in a forced-air drying oven at 48° for a minimum of 72 hr. Ninety-three grams of a 40-mesh powder of the whole mushroom was extracted exhaustively with successive portions of petroleum ether, diethyl ether, chloroform, and 95% ethanol in a Soxhlet apparatus. An orange-red crystalline precipitate (6.33 Gm.) separated from the ether solution during extraction and was removed by filtration prior to evaporation of the solvent. Gripenberg (6) had isolated aurantiacin from a similar precipitate obtained upon ether extraction of *Hydnellum aurantiacum* (Fr.) Karst. (*Hydnum aurantiacum* Batsch); consequently, this precipitate was selected as the first fraction to be examined.

Chromatographic Evaluation of the Crude Precipitate.—Chromatography was potentially the most convenient way to obtain a preliminary evaluation of the various extracts, but no chromatographic procedures have been reported for the separation of diphenylbenzoquinone compounds. Thus, a solution of the precipitate from the ether extract was examined using a variety of adsorbents and solvent systems to determine if it could be resolved readily. Maximum resolution of the components in the crude precipitate was obtained on a layer of Silica Gel G with a benzene–ethyl acetate–glacial acetic acid (75:24:1) solvent system. A mixture of 20 Gm. of Silica Gel G and 40 ml. of distilled water was shaken for 1 min. and spread on 20 × 20 cm. glass plates in a uniform 250-μ layer. The plates were allowed to stand for 10 min., heated at 105–110° for 30 min., and cooled. The crude material was applied to the chromatograms, and they were formed ascendingly using the solvent mixture. Some material remained at the point of origin; the other components separate into five chromatographic zones, two of which developed latent colors upon standing at room temperature for 24–48 hr. The actual migration of the zones on the chromatographic layers was observed to vary slightly with the quantity of material spotted and with different batches of chromatographic plates. However, the relative position of the zones was always the same, and representative R_f values are as follows: 0.33, yellow-orange; 0.24, latent lavender-brown; 0.17, yellow-brown; 0.13, latent gray; 0.10, slate gray. The ether filtrate which remained after separation of the precipitate exhibited the same qualitative composition upon chromatography. Examination of available diphenylbenzoquinones revealed that atromentin and polyporic acid remained at the point of origin under these chromatographic conditions, whereas 2,5-diphenyl-1,4-benzoquinone had an R_f value of 0.63.

Isolation of the Major Component of the Crude Precipitate.—Gripenberg (6) utilized a column of acid-washed alumina in his isolation of aurantiacin, but the results obtained with thin-layer chromatography and with preliminary columns suggested that the use of silica gel permitted more effective separation of the components of *H. caeruleum.* A mixture of silica gel (100–200 mesh) and flux calcined diatomaceous silica[1] (75:1) was suspended in benzene and transferred to an 18-mm. diameter extrusion-type chromatographic column to give a 30.5-cm. column of adsorbent. A sample of the crude precipitate (1.3 Gm.) was dissolved in a minimum volume of dioxane and adsorbed on a small quantity of the silica gel–flux calcined diatomaceous silica[1] mixture. The dioxane was evaporated at 50°, and the adsorbed material was added to the top of the chromatographic column. The column was washed successively with approximately 2000 ml. of benzene–ethyl acetate (9:1), 1850 ml. of benzene–ethyl acetate (1:1), 150 ml. of ethyl acetate, and 400 ml. of ethyl acetate–glacial acetic acid (20:1). The eluate was collected in approximately 50-ml. fractions, and 25–50-μl. portions of each fraction were examined using the thin-layer chromatographic procedure. The first 200 ml. of eluate contained no pigmented compounds, and the next 900 ml. were chromatographically homogeneous (R_f 0.33). Thin-layer chromatography revealed that the compound with R_f 0.33 was the major component of the crude precipitate; it was detected in all subsequent fractions of the eluate. The eluates containing only the one compound (R_f 0.33) were combined, the solvent was evaporated in a stream of air at room temperature, and the residue was recrystallized 3 times from dioxane. The solvent of crystallization was removed by heating in a drying pistol over phosphorus pentoxide at 146° *in vacuo* for 120 hr.

Isolation of the orange-red crystalline material using the column chromatographic procedure was tedious and relatively inefficient. Consequently, an alternate procedure which employed selective solubilization of the accompanying impurities was developed. A sample of the crude precipitate (2.5 Gm.) was stirred with successive 50-ml. portions of methanol, and the supernatant solutions were examined using thin-layer chromatography. After washing the crude precipitate with 5 portions of methanol, the remaining residue was chromatographically homogeneous (R_f 0.33). When this material was recrystallized from dioxane and dried, it was indistinguishable from the crystalline substance obtained by column chromatography.

Preliminary Identification of the Isolated Material.—Qualitative elemental analysis following sodium fusion revealed the absence of nitrogen, sulfur, and halogens in the molecule. The infrared spectrum of the compound in a potassium bromide pellet using a Beckman infrared spectrophotometer, model IR5A, strongly suggested the presence of an

[1] Marketed as Hyflo Super Cel by the Johns-Manville Corp.

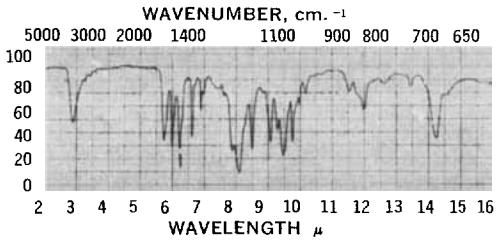

Fig. 1.—Infrared spectrum of isolated aurantiacin.

ester carbonyl group (1748 cm.$^{-1}$) and a quinone carbonyl function (1665 cm.$^{-1}$). The balance of the infrared spectrum (Fig. 1) was consistent with that anticipated for a substituted diphenylbenzoquinone compound (24), and the micro m.p., K., of 285–295°, agreed with the melting point reported for aurantiacin (6).

Visible and ultraviolet absorption spectra are useful in distinguishing some diphenylbenzoquinones (25). Such spectra were obtained by dissolving 2.0 mg. of the recrystallized compound in 10 ml. of dioxane, diluting the solution 25-fold, and recording the absorption with a Beckman spectrophotometer, model DB. The absorption maxima obtained at 240 mμ (log ϵ = 4.71) and 405 mμ (log ϵ = 3.94) were in agreement with the values 240 mμ (log ϵ = 4.63) and 405 mμ (log ϵ = 3.82) reported for aurantiacin.

Degradation of the Isolated Material.—Preliminary data suggested that the isolated material was aurantiacin, a compound which can be decomposed to atromentin and benzoic acid (6). A small sample of the crystals (100 mg.) was dissolved in 50 ml. of 2 *N* sodium hydroxide solution. The alkaline solution was acidified with concentrated hydrochloric acid and partitioned with three successive 50-ml. portions of ether. The ether solution was extracted with three 50-ml. portions of saturated sodium bicarbonate solution. Acidification of the bicarbonate solution gave a brown precipitate which was separated by filtration and washed with benzene. The mother liquor from the filtration was extracted with ether, the ether was removed by evaporation, and the residue was washed with benzene. The corresponding benzene-soluble and insoluble portions of the precipitate and residue were combined.

The benzene solution was evaporated to dryness, and the residue was sublimed under reduced pressure. Micro m.p., K., of the sublimate was 120–121°. An admixture with authentic benzoic acid showed no depression in melting point.

The benzene–insoluble material was recrystallized from methanol and dried *in vacuo* over phosphorus pentoxide at 146° for 120 hr. It decomposed without melting at about 303–305°. A dioxane solution of the material (1 mg. dissolved in 10 ml., then diluted 25-fold) had absorption maxima at 268 mμ (log ϵ = 4.51) and 384 mμ (log ϵ = 3.73); these results agreed with the values 268 mμ (log ϵ = 4.55) and 385 mμ (log ϵ = 3.66) reported for atromentin (25).

DISCUSSION AND CONCLUSIONS

Extraction and fractionation procedures were utilized to investigate the occurrence of diphenylbenzoquinone compounds in carpophores of *H. caerulum*. An orange-red crystalline compound was isolated from a precipitate that separated from the ether extract. The melting point and spectral properties (infrared, visible, and ultraviolet) of the isolated compound corresponded to those of aurantiacin. This identification was confirmed by alkaline degradation of the material to atromentin and benzoic acid.

The isolation of aurantiacin from *H. caeruleum* marks the second reported occurrence of this compound in nature and the first since its original isolation from *H. aurantiacum* (6). The genus *Hydnellum* is a reasonably well-defined taxon which is characterized by a tough fibrous or leathery context (26). Major morphologic criteria for the separation of species within the genus are the presence or absence of clamp connections in the hyphae of the pileal trama and the presence or absence of incrusting granules in the tramal hyphae. *H. aurantiacum* has incrusting granules and no clamp connections; *H. caeruleum*, the antipode, has clamp connections and no granules. Thus, morphologic considerations suggest that the two species are not as closely related as could be predicted on the basis of aurantiacin accumulation. However, there is no available evidence which permits the assignment of a greater or less phylogenetic significance to chemotaxonomic indicators than to morphologic relationships.

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