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SYNTHESIS OF JUGLONE DERIVATIVES
HYDROXY, ACETYL AND ETHYL SUBSTITUENTS

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Abstract—A number of hydroxyjuglones were prepared by the Thiele-Winter reaction on juglone derivatives and by hydroxylation with ethanol-hydrochloric acid: 2-hydroxy, 3-hydroxy, 2,7-dihydroxy, 3,7-dihydroxy, 6-acetyl-2-hydroxy, and 6-acetyl-3-hydroxyjuglone. By reductive acetylation of hydroxyjuglones, followed by Fries rearrangement and oxidative hydrolysis several acetyljuglones, and from them ethyljuglones by sodium borohydride reduction, were synthesized: 3-acetyl-2-hydroxy, 6-acetyl-2,3-dihydroxy, 6-acetyl-2,7-dihydroxy, and 6-acetyl-2,3,7-trihydroxyjuglone. Several of the newly synthesized compounds had previously been isolated from sea urchin spines.

DERIVATIVES of juglone (5-hydroxy-1,4-naphthoquinone) occupy a unique place in quinone chemistry. Although in a formal sense juglone is halfway between 1,4-naphthoquinone and naphthazarin (5,8-dihydroxy-1,4-naphthoquinone), it represents a unique molecular system lacking the simplicity of naphthoquinone and the symmetry of naphthazarin. The phenolic 5-OH group sensitizes the molecule toward alkali and oxidizing agents and at the same time gives rise to a large number of closely related positional isomers. The number of reported naturally occurring juglone derivatives is small. Thomson's authoritative monograph,[1] published in 1957, describes fewer than ten such compounds. One of these, 2,3,7-trihydroxyjuglone, is the sea urchin pigment spinochrome N, which in 1964 was shown by Gough and Sutherland[2] to be identical with the previously isolated spinochromes B, B_1, M_2, and P_1. It was the only juglone derivative among the characterized sea urchin pigments; all others were derivatives of naphthazarin. In 1966 we reported the isolation and identification of four new juglones from the spines and shells of two sea urchin species of the genus *Echinothrix*.[3] The new pigments were 2-hydroxy-6-ethyl, 2,7-dihydroxy-6-acetyl, 2,3,7-trihydroxy-6-ethyl, and 2,3,7-trihydroxy-6-acetyljuglone. Sutherland in 1967[4] identified yet another new juglone, 2,6,7-trihydroxy-3-acetyljuglone from an Australian sea urchin, *Salmacis sphaeroides*.

A majority of these pigments occur in nature in exceedingly low concentration. It was therefore desirable to attempt their synthesis in order to facilitate a study of their physical and chemical properties. Since substituents of typical echinoderm pigments seem to be restricted to OH (or OMe) and two-carbon side chains (ethyl or acetyl),[5] we concentrated our efforts on the synthesis of juglone derivatives bearing those substituents.

Previous research in this area dates back to 1885, when Mylius[6] prepared hydroxyjuglones by direct oxidation of juglone in basic medium and by acid hydrolysis of a dimethylamino derivative. He believed the two hydroxyjuglones to be identical, but did not know whether he had the 2- or 3-hydroxyjuglone in hand. More than 60 years later Thomson[7] showed that Mylius' hydroxyjuglone derived from the dimethylamino compound was the 2-hydroxy, while his direct oxidation product was

the 3-OH compound. Cooke *et al.*[8] showed that both 2- and 3-dimethylamino compounds are initially formed from juglone methyl ether, but on hydrolysis only 2-hydroxyjuglone can be isolated.

Garden and Thomson[9] prepared 3,6-dihydroxyjuglone by the lengthy tetralone route and from it, by reduction with stannous chloride, as described by Bruce and Thomson,[10] these authors synthesized 6-hydroxyjuglone. Smith and Thomson[11] elaborated their general procedure to the synthesis of 2,3,7-trihydroxyjuglone (spinochrome N, *alias* B, *vide supra*). Natori and Kumada[12] reported the synthesis of 3,6,7-trihydroxyjuglone by the tetralone route and of 2,6,7-trihydroxyjuglone by a much shorter path which had been developed by Bycroft and Roberts[13] for the synthesis of 2,7-dihydroxyjuglone (flaviolin). In this procedure ring closure of a suitably substituted benzene leads directly to a hydroxynaphthalene, which can be oxidized to a quinone. The Bycroft and Roberts synthesis was further improved and simplified by Baker and Bycroft[14] in an elegant synthesis of flaviolin.

Birch's[15] Diels–Alder reaction of benzoquinone and dimethoxycyclohexadiene has led to the dimethyl ether of 7-hydroxyjuglone, but the versatility of this method for a general synthesis of substituted juglones is limited by the availability of suitable cyclohexadienes.

Only two syntheses of ethyl-substituted juglones have been reported in the literature. Cooke and Segal[16] prepared 2-hydroxy-3-ethyljuglone methylether from 5-methoxy-1-tetralone as an intermediate in their synthesis of 3-hydroxy-2-methyljuglone (droserone). Also from a tetralone precursor, Hase[17] prepared 2,6,7-trihydroxy-3-ethyljuglone.

Reports on acetyl substituted juglones are equally sparse. Spruit[18] prepared 6-acetyljuglone from 1,5-dihydroxynaphthalene by a Fries reaction, followed by diazo coupling, reduction to the amine, and oxidation to the quinone. Cooke and Sparrow[19] obtained a mixture of 3-acetyl-2-methyl- and 6-acetyl-7-methyljuglone by oxidation of 2-acetyl-3-methyl-1,8-dihydroxynaphthalene with Fremy's salt.

In our own laboratory[20] we obtained a number of ethyl- and acetylhydroxyjuglones by the sodium borohydride reduction of 2,7-dihydroxy-3-acetylnaphthazarin (spinochrome A), but none in anything approaching preparative yields—not to mention the scarcity of starting material. As part of our structure proof of one compound we synthesized 2-hydroxy-3-ethyljuglone from 2-hydroxyjuglone and propionylperoxide in analogy with Cooke and Segal's[16] earlier work.

Hydroxylation reactions. When juglone is treated with acetic anhydride-sulfuric acid (Thiele–Winter reaction), followed by oxidation in basic solution, a mixture of 2-hydroxy (42%) and 3-hydroxyjuglone (21%) is obtained which is readily separable by preparative TLC. By following the procedure of Fieser and Dunn,[21] who did not separate the mixture, we obtained the two hydroxyjuglones in yields of 37 and 26%. Thomson[7] had earlier repeated the Thiele–Winter reaction on juglone as described by Fieser[21] and had reported the product to be 3-hydroxyjuglone. Clearly, the intermediate leucoacetate must be a mixture of 1,2,4,5- and 1,3,4,5-tetraacetoxynaphthalenes. Both Fieser and Thomson report the m.p. of the intermediate to be 154°. We prepared both leucoacetates by reductive acetylation of pure 2- and 3-hydroxyjuglone and obtained two compounds with the same m.p., 161–162°. A mixture m.p., however, was 140–154°. Since 3-hydroxyjuglone is less soluble than its 2-isomer, it is reasonable that spontaneous fractionation during crystallization of the hydrolysis mixture would yield the 3-isomer.

Further hydroxylation of 2- or 3-hydroxyjuglone by the Thiele–Winter reaction fails, even under more vigorous conditions. Only starting material is recovered.

Attempts to prepare 7-hydroxyjuglone from maleic anhydride and resorcinol diacetate or resorcinol dimethyl ether under Zahn–Ochwat[22] conditions were unsuccessful. The isolated products were carboxylic acids and not juglones. On the basis of spectral data and in analogy with the work of Barr *et al.*[23] these compounds were normal Friedel–Crafts products and had the general structure $ArCOCH{=}CHCO_2H$. An attempted synthesis of 6,7-dihydroxyjuglone from pyrogallol triacetate and maleic anhydride also yielded products which were not juglones. 7-Hydroxyjuglone was synthesized by Garden and Thomson's[9] method and subjected to the Thiele–Winter reaction. The reaction mixture was separated by TLC into 2,7-dihydroxyjuglone (flaviolin) as the major (69%) and 3,7-dihydroxyjuglone as the minor (10%) product. Clean separation of the two isomers can only be achieved by repeated chromatography on deactivated silica gel plates and is preferably carried out after methylation with excess diazomethane. The mixture of 2-methoxy and 3-methoxy-7-hydroxyjuglone is readily separable by preparative TLC and the parent compounds can be quantitatively recovered by refluxing in ethanol–hydrochloric acid under nitrogen.

Further hydroxylation of juglone was also attempted by use of ethanol and hydrochloric acid. The reaction led to starting material (10%), 2-hydroxy (5%), and 3-hydroxyjuglone (26%). Although the 3-isomer is the major product, the absolute yields in this reaction are inferior to those in the Thiele–Winter reaction.

No additional OH groups could be introduced into 2-hydroxy or 7-hydroxyjuglone by this method. Trace amounts of 3,7-dihydroxyjuglone could be recovered from the reaction on the 7-hydroxy isomer and extensive decomposition was evident.

Hydroxylation by the ethanol-hydrochloric acid method was somewhat more successful with 6-acetyljuglone, which we prepared by Spruit's[18] procedure. Separation of the reaction mixture by TLC furnished 3-hydroxy-6-acetyljuglone as the major product (22%) in addition to 2-hydroxy-6-acetyljuglone (5%), 3-hydroxyjuglone (9%), and recovered starting material (25%). No ready explanation is apparent for generation of a deacetylated product in this reaction. Preferential attack at C-3 by water or ethanol would be expected if one assumes that the non-H bonded C-1 CO is protonated thus leading to a carbonium ion at C-3.

Further hydroxylation of juglone and hydroxyjuglones is clearly difficult. We have shown that it can be achieved in selected cases under Thiele–Winter conditions. The more drastic ethanol–hydrochloric acid method succeeds only when a stabilizing group as e.g. acetyl is present.

Acetylation reactions. Since our objective in this work was the synthesis of juglone derivatives bearing additional hydroxy as well as Ac (or Et) substituents, and since introduction of OH groups is severly limited, we turned next to the problem of acetylation. Because of the sensitivity of the juglone system it is not feasible to introduce Ac directly into a juglone derivative. Instead, acetylation has to be carried out on a reduced, suitably substituted naphthalene. Based on this premise we have reductively acetylated a number of juglone derivatives to their leucoacetates; introduced an acetyl group generally by treatment with acetic anhydride–boron trifluoride (Fries rearrangement); and regenerated the juglone system by oxidative hydrolysis.

Juglone was converted to 1,4,5-triacetoxynaphthalene with acetic anhydride, zinc, and fused potassium acetate in 87% yield. Subsequent Fries reaction and hydrolysis

went poorly and led to a mixture of 6-acetyljuglone, 2-hydroxy-3-acetyljuglone in addition to several unidentified compounds. We also prepared 6-acetyljuglone by Spruit's[18] procedure, which involves acetylation of 1,5-dihydroxynaphthalene; coupling of the acetylnaphthalenediol with diazotized sulfanilic acid; reduction of the diazonium salt to 6-acetyl-4-amino-1,5-dihydroxynaphthalene; and ferric chloride oxidation to the juglone. Separation of the reaction product by preparative TLC showed that small amounts of 2- and 3-hydroxy-6-acetyljuglone are also formed during this reaction sequence. 2-Hydroxy-3-acetyljuglone was reduced with sodium borohydride to 2-hydroxy-3-ethyljuglone, identical in all respects with an authentic sample obtained by us[20] by sodium borohydride reduction of spinochrome A.

From 2-hydroxyjuglone we obtained by the same reaction sequence a 93% yield of leucoacetate and from it 2-hydroxy-6-acetyljuglone (48%), 2-hydroxy-3-acetyljuglone (5%), and starting material (5%).

3-Hydroxyjuglone led to the leucoacetate in 88% yield. From it we obtained as the major product 3-hydroxy-6-acetyljuglone as well as small amounts of 2-acetyl-3-hydroxy and 3-hydroxyjuglone.

For the synthesis of 2,3-dihydroxyjuglone we followed Garden and Thomson's[9] published procedure. Juglone methyl ether had the expected NMR spectrum and epoxidation proceeded smoothly. We could not, however, verify the observation that the epoxide precipitated from solution. In our hands, the precipitate proved to be inorganic and the epoxide was extracted from the solution with ether. The epoxide, the 2,3-diacetoxy, and the 2,3-dihydroxyjuglone methyl ether all had the expected NMR spectra. The final step in the synthesis, cleavage of the methyl ether, proceeded better with 48% hydrobromic acid under nitrogen than with Thomson's[9] aluminum chloride–sodium chloride mixture. Thomson's[9] UV data for 2,3-dihydroxyjuglone (397, 567 nm) differ widely from ours (248, 275 sh, 403, 485 sh nm). Our UV data are those of a typical juglone derivative,[24] while a long wavelength band at 567 nm seems unprecedented.* Cooke and Owen[25] had also synthesized this compound by a different route, but reported no UV data.

Acetylation of 2,3-dihydroxyjuglone proceeded well. By our usual procedure we prepared 1,2,3,4,5-pentaacetoxynaphthalene in 88% yield and from it we obtained 6-acetyl-2,3-dihydroxyjuglone (87%) as the sole product since only one Fries rearrangement is possible. Sodium borohydride reduction of the acetyl compound led to 6-ethyl-2,3-dihydroxyjuglone (41%) accompanied by a small amount of 6-ethyl-3-hydroxyjuglone and by recovered starting material (27%). On prolonged treatment with sodium borohydride the yield of 6-ethyl-dihydroxy compound is reduced and that of 6-ethyl-3-hydroxy compound is increased. We had previously noted[20] the loss of hydroxy groups during sodium borohydride reduction in polyhydroxynaphthoquinones. We have now observed that reduction of acetyl to ethyl proceeds poorly when the quinoid ring has no or only one substituent. Methylation of 6-ethyl-2,3-dihydroxyjuglone with diazomethane led to the expected 2,3-dimethyl ether.

2,3,7-Trihydroxyjuglone (spinochrome B) was reductively acetylated to yield 1,2,3,4,5,7-hexaacetoxynaphthalene. A Fries reaction on this compound, followed by the usual work-up, furnished 6-acetyl-2,3,7-trihydroxyjuglone, which on treatment with diazomethane led to 6-acetyl-2,3-dimethoxy-7-hydroxyjuglone, identical in all

* Prof. Thomson has remeasured the UV spectrum of his sample of 2,3-dihydroxyjuglone. He has informed us that the spectrum agrees with ours and that his previously published spectrum[9] is wrong.

respects with the dimethyl ether of a natural pigment which we had isolated from the sea urchin *Echinothrix diadema*.[3]

2,7-Dihydroxyjuglone (flaviolin) was reductlively acetylated to 1,2,4,5,7-pentaacetoxynaphthalene and subjected to a Fries rearrangement. Separation of the product mixture by TLC led to 6-acetyl-2,7-dihydroxyjuglone, identical with a natural pigment from *E. diadema*;[3] to 3-acetyl-2,7-dihydroxyjuglone, identical with a natural pigment from *E. diadema* not previously reported; and an unidentified trace substance.

Acetyljuglones, and by sodium borohydride reduction, ethyljuglones can be readily prepared by Fries reaction of polyacetoxynaphthalenes. This route generally leads to a mixture of acetyljuglones unless substitution is such that only one Fries reaction can take place.

EXPERIMENTAL

All m.ps were determined on a Fisher–Johns block and are uncorrected. All IR spectra were measured on a Beckman IR-5 automatic recording spectrophotometer using NaCl cells unless otherwise specified. All UV and visible spectra were recorded on a Cary 14 recording spectrophotometer. $CHCl_3$ was the neutral solvent unless specified otherwise. NMR spectra were observed on a model A-60 Analytical NMR Spectrometer (Varian Associates) or, if specified, on a model HA-100 (Varian). All values are expressed in ppm referred to TMS having a value of 0. Mass spectra were recorded on a Hitachi–Perkin–Elmer RMU-6D mass spectrometer. Separation of the juglone derivatives and their purification was achieved using column chromatography and TLC. Adsorbent for column chromatography was acid-deactivated silica gel (dsg) which was prepared as follows: silica gel powder (chromatographic grade) was washed with 0·5N HCl and dried with suction. The resulting dsg was spread on a porcelain tray and air-dried. Some dsg was also dried with the aid of a heat lamp, but care must be taken not to drive off the acid. The plates for TLC were prepared using a Desaga/Brinkmann standard applicator (Brinkmann Instruments, Inc.). A slurry of 2·' mixture of 0·5N HCl and silica gel G (E. Merck, Germany) was stirred in a mortar and applied to a ies or combination of plates (20 × 20 and/or 20 × 5 cm) which were then allowed to dry at room temp or at least 6 hr. Usually a thickness of 0·25 mm was used for initial spot testing and a thickness of 0·5 mm was used for preparative TLC. Benzene was the eluant unless specified.

Thiele reaction on juglone. The Thiele reaction was carried out by dissolving 50 mg juglone in 20 ml Ac_2O and adding ca. 1 ml conc H_2SO_4. The color of the soln faded. It was kept at rt for 1 hr during which it turned dark. After decomposition over ice it was extracted with ether. The ether was stripped and the residue was heated with dil HCl for about 15 min. It was cooled, extracted with ether, and oxidized by shaking with NaOH aq for a few sec. The aqueous extract was poured into ice–HCl and extracted with ether. The ether extract was dried over Na_2SO_4 and the ether was evaporated. The residue was analyzed by TLC. The fastest moving band contained 24 mg 2-hydroxyjuglone (42%). The second band contained 12 mg 3-hydroxyjuglone (21%).

Thiele reaction on juglone (after Fieser and Dunn[21]). With warming 100 mg (0·58 mmole) freshly sublimed juglone was dissolved in 2 ml Ac_2O. Two drops conc H_2SO_4 were added and the soln was kept at rt for 10 d. After decomposition over ice the mixture was extracted with ether, the ether was removed and the residue was heated with dil HCl for ca. 15 min. The mixture was cooled, extracted with ether, and was air-oxidized by shaking with NaOH aq for a few seconds. The aqueous layer was poured into ice-HCl and extracted with ether. The ether extract was dried over Na_2SO_4 and the ether removed. The residue contained 40 mg 2-hydroxyjuglone (37%) and 28 mg (26%) 3-hydroxyjuglone.

1,2,4,5-*Tetraacetoxynaphthalene.* A soln of purified 2-hydroxyjuglone (370 mg) in Ac_2O (30 ml) was refluxed with Zn powder (500 mg) and fused KOAc (200 mg) for 5 hr. The reaction mixture was filtered hot and the residue was washed with hot Ac_2O. The solvent was removed from the filtrate. The oily residue on washing with water furnished a solid (650 mg, 93%) which was crystallized from EtOH, white needles, m.p. 161–162°; NMR spectrum in acetone-d_6: C-1—OAc, δ 2·47 (s, 3H); C-2—OAc, δ 2·31 (s, 3H); C-4 and C-5—OAc, δ 2·40 (s, 6H); C-3, C-6, C-7, C-8—H, δ 7·20–8·06 (m, 4H).

1,3,4,5-*Tetraacetoxynaphthalene* was obtained by the reductive acetylation of purified 3-hydroxyjuglone in 88% yield. It was crystallized from EtOH, white needles, m.p. 161–162°. On admixture, it depressed the m.p. of the isomeric 1,2,4,5-tetraacetoxynaphthalene, 140–154°; NMR spectrum in acetone-d_6: C-1—OAc, δ 2·47 (s, 3H); C-3—OAc, δ 2·33 (s, 3H); C-4—OAc, δ 2·44 (s, 3H), C-5—OAc, δ 2·40 (s, 3H), C-2, C-6, C-7, C-8—H, δ 7·03–7·95 (m, 4H).

Thiele reaction on 7-hydroxyjuglone. 7-Hydroxyjuglone was prepared by the procedure of Garden and Thomson.[9] A Thiele reaction by our method led to a mixture which was separated by TLC using $CHCl_3$ as the eluant.

Band 1, 2,7-dihydroxyjuglone (flaviolin), (21 mg, 69%) crystallized as orange needles from chloroform : isooctane (1 : 1), m.p. 255–258° (dec) (lit[13] 250° dec); UV spectrum, $\lambda_{max}^{CHCl_3}$ 422 (broad), 398 (broad), 305, 260 nm. R_f value and electronic spectrum were identical with a sample of flaviolin obtained from Dr. J. C. Roberts.

Band 2, 2 mg (10%) was found to be identical with 3,7-dihydroxyjuglone.[24]

7-Hydroxy-2-methoxyjuglone. 2,7-Dihydroxyjuglone was dissolved in ether and treated with a small excess of diazomethane in ether. The soln was evaporated immediately under vacuum and the residue on purification through TLC gave 2-methoxy-7-hydroxy-juglone, crystallized from pet. ether (30–60°) as orange needles, m.p. 220–221°; NMR spectrum in $CDCl_3$: C-2—OCH-OCH_3, δ 3·8; UV spectrum $\lambda_{max}^{CHCl_3}$ 438, 298, 260 nm.

Hydroxylation of juglone using EtOH–HCl. A soln of 50 mg juglone in 10 ml 95% EtOH was boiled gently and 10 ml conc HCl was added dropwise over a 15 min period. There was some separation of a green decomposition product. The reaction mixture was cooled and extracted with ether. The ether extract was dried over Na_2SO_4 and the ether was evaporated. The residue was analyzed by TLC. Three yellow bands were isolated. The fastest moving band was juglone, 5 mg. The second band contained 3 mg 2-hydroxyjuglone (5%). The third band contained 15 mg (26% yield) 3-hydroxyjuglone.

6-Acetyljuglone. The preparation was based on Spruit's[18] procedure. 1,5-Dihydroxynaphthalene (10 g) was refluxed in 30 ml HOAc and 30 ml $BF_3 \cdot OEt_2$ for 30 min. After addition of water the mixture was allowed to sit while long needles formed. The solid was filtered, washed with water, redissolved in excess MeOH, filtered again and water was slowly added to the hot solution until cloudiness appeared. Upon cooling a black tarry solid was filtered off. Concentration of the mother liquor gave a yellow-green solid, m.p. 268–270° (lit[18] m.p. 265°). The mass spectrum indicated a mo. wt of 202 with a small amount of impurity from the diacetyl derivative. NMR spectrum (acetone–d_6): C-2 Ac, δ 2·73 (s, 3H); C-3, C-4, C-6, C-7 and C-8 H's, 7·0–8·0 (m, 5H). Diazobenzenesulfonic acid (3·8 g) was prepared. With N_2 bubbling through a soln of 3 g NaOH and 17 ml H_2O which was cooled with 10 g ice, 2·76 g 2-acetyl-1,5-dihydroxynaphthalene was added. With external cooling and vigorous stirring diazobenzenesulfonic acid was added in small amounts. After stirring for 5 min the deep red-purple mixture was allowed to sit at rt for 15 min. Upon warming to 60°, 6 g $Na_2S_2O_4$ was added with stirring for 10 min. A lot of foam was formed. The mixture was filtered through a Buchner funnel; the filtrate was later evaporated and treated like the filtered solid. The solid was carefully washed into a beaker using 100 ml H_2O. Much of the solid dissolved in the water. A pinch of $SnCl_2$ and 3–4 ml conc HCl was added. The mixture was heated to the b.p.; conc HCl (3 ml) was added and the mixture was cooled to rt and filtered. The resulting reddish solid was placed in a Soxhlet extractor attached to a 4 l. flask and extracted with H_2O. A small amount of $SnCl_2$ was added to the flask during extraction to prevent oxidation. To the hot solution was added 17 g $FeCl_3 \cdot 6H_2O$ in 25 ml H_2O and 25 ml conc HCl. The solution was immediately cooled. The ppt was collected, followed by extraction of the H_2O soln with $CHCl_3$. After drying and removal of solvent both solids were dissolved in $CHCl_3$ and placed on a column. The first yellow-orange band contained almost all of the 6-acetyljuglone. Separation by TLC and crystallization from $CHCl_3$-isooctane gave a cryst solid, m.p. 125–127°; UV spectrum; λ_{max} 240 and 418 nm; NMR spectrum ($CDCl_3$): C-2 and C-3 H's, δ 7·01 (s, 2H); C-5 OH, 12·96 (s, 1H); C-6 Ac, 2·73 (s, 3H); C-7 H, 8·15 (d, J = 8 Hz, 1H); C-8 H, 7·65 (d, J = 8 Hz, 1H). The mass spectrum gave a molecular ion of m/e 216.

Also isolated from the reaction mixture were two slower moving compounds. The faster moving of the two bands was rechromatographed and was shown to be soluble in dil $NaHCO_3$; UV spectrum: λ_{max} 240, 290 and 425 nm. Its R_f value was identical with that of an authentic sample of 6-acetyl-2-hydroxyjuglone.[20] The UV spectra were also superimposable. The second band was identical with band 4 from the EtOH–HCl hydroxylation of 6-acetyljuglone (*vide infra*). The R_f values and UV spectra were identical. UV spectrum: λ_{max} 238, 282 and 418 nm; NMR spectrum ($CDCl_3$): C-2 H, δ 6·40 (s, 1H); C-5 OH, 12·62 (s, 1H); C-6 Ac, 2·75 (s, 3H); C-7 H, 8·22 (d, J = 8 Hz, 1H); C-8 H, 7·74 (d, J = 8 Hz, 1H).

Hydroxylation of 6-acetyljuglone with EtOH–HCl. 6-Acetyljuglone (60 mg) was dissolved in 20 ml EtOH. To the boiling soln 25 ml conc HCl was added over a 30 min period. A green ppt separated after boiling for 30 min longer. The reaction mixture was cooled, diluted with water and extracted with ether. The ethereal extract was dried over anh Na_2SO_4 and the ether was evaporated. The residue was subjected to TLC.

Band 1 contained 15 mg 6-acetyljuglone. Band 2 contained 6 mg 3-hydroxyjuglone which had an R_f value identical with that of an authentic sample. The UV spectrum had λ_{max} 240, 284 and 420 nm and was superimposable with that of an authentic sample. Band 3 contained 4 mg 6-acetyl-2-hydroxyjuglone. Its

R_f value and the UV spectrum were identical with those of an authentic sample. The UV spectrum had λ_{max} 240, 290, and 425 nm.

Band 4 was the major product, 14 mg. After recrystallization from $CHCl_3$-isooctane, it melted 164–166°. The UV spectrum of 6-acetyl-3-hydroxyjuglone had λ_{max} 239, 279 and 418 nm. The NMR spectrum ($CDCl_3$) exhibited a signal for the C-2 H, δ 6·38 (s, 1H); C-5 OH, 12·63 (s, 1H); C-6 Ac, 2·75 (s, 3H); C-7 H, 8·21 (d, J = 8 Hz, 1H); C-8 H, 7·72 (d, J = 8 Hz, 1H). The mol wt of 232 was confirmed by the mass spectrum.

Fries reaction on juglone leucoacetate. Freshly sublimed juglone (1 g) in 12 ml Ac_2O was refluxed with 500 mg Zn dust and 100 mg fused KOAc for 3–4 hr. The yellow soln was filtered hot and washed with Ac_2O. After removal of Ac_2O a green-yellow solid, m.p. 118–120°, remained. Recrystallization from EtOH gave 1·52 g (87%) 1,4,5-triacetoxynaphthalene. NMR ($CDCl_3$) signals: C-1 acetoxy, δ2·42 (s, 3H); C-4 and C-5 acetoxy, 2·36 (s, 6H); C-2, C-3, C-6, C-7 and C-8 hydrogens, 7·0–7·9 (m).

Juglone leucoacetate (100 mg) was dissolved in 5 ml Ac_2O and BF_3 was bubbled through for 3–4 min. The temp rose to 85°. The soln changed to yellow, then to green-yellow, and finally to orange-red. The volume was reduced under vacuum as the solution turned darker. Upon cooling and decomposition with ice water an orange solid was collected. The solid was dissolved in 20 ml 1N NaOH under N_2. The solution was refluxed under N_2 for 1 hr, cooled, shaken with air for a few sec, and poured into ice–HCl. Extraction with ether, drying and removal of ether followed. The residue was placed on TLC plates and separated into four main bands.

Band 1 contained only traces of yellow compounds. The red compounds noted were probably traces of naphthazarin and acetylnaphthazarin. No further work was done on these compounds.

Band 2, the second fastest moving band, was again purified by TLC and crystallized from $CHCl_3$–isooctane giving a yellow solid, m.p. 161–162°. UV spectrum λ_{max} 284 and 405 nm. The mass spectrum gave the mol wt as 232, which corresponds to an acetyl juglone plus one OH group. The assigned structure was 3-acetyl-2-hydroxyjuglone. NMR spectrum ($CDCl_3$): C-3 Ac, δ 2·85 (s, 3H); C-5 OH, 12·68 (s, 1H); C-6, C-7 and C-8 H's, 7·3–7·85 (m, 3·5H).

Upon reduction with $NaBH_4$ in MeOH followed by hydrolysis and oxidation with base and shaking with air for a few sec, the reduced band 2 mixture was poured into ice–HCl and extracted with ether. The ether was dried and evaporated. By TLC it was determined that 3-ethyl-2-hydroxyjuglone was formed as it had an R_f value identical with that of an authentic sample.[20]

Ahead of band 3 moved a very small band which was not isolated. Band 3 was rechromatographed using CCl_4 and recrystallized from $CHCl_3$–isooctane to give orange plates, m.p. 120–122°, 6-acetyljuglone. Lit.[18] m.p. 127°.

The R_f values in benzene and $CHCl_3$ indicated it was a single compound. The R_f value was the same as for 6-acetyljuglone prepared by the Spruit method. UV spectrum: λ_{max} 235, 277 sh and 418 nm.

Band 4, as shown by the NMR spectrum, consisted of unhydrolyzed acetates. Refluxing in NaOH aq under N_2 for 30 min provided more of bands 1, 2 and 3.

Fries reaction on 2-hydroxyjuglone leucoacetate. By the general procedure outlined above 2-hydroxyjuglone (370 mg) was converted to 1,2,4,5-tetraacetoxynaphthalene, white needles, m.p. 161–162° (650 mg, 93%); NMR spectrum (acetone–d_6): C-1 OAc, δ 2·47 (s, 3H); C-2 OAc, 2·31 (s, 3H); C-4 and C-5 OAc, 2·40 (s, 6H); C-3, C-6, C-7 and C-8 H's, 7·20–7·95 (m, 4H). The mass spectrum confirmed the mol wt of 360. When mixed with 1,3,4,5-tetraacetoxynaphthalene, the mixture m.p. was 140–154°. The Fries reaction on 1,2,3,5-tetraacetoxynaphthalene (200 mg) furnished after TLC separation 3-acetyl-2-hydroxyjuglone, m.p. 158–159° from $CHCl_3$–isooctane, 12 mg (5%); 2-hydroxyjuglone, 13 mg (4%); and 6-acetyl-2-hydroxyjuglone, m.p. 191–193° (dec) 111 mg (48%) from $CHCl_3$–isooctane, identical with a product obtained by $NaBH_4$ reduction of spinochrome A,[20] UV spectrum: λ_{max} 240, 290 and 425 nm; NMR spectrum (acetone–d_6): C-3 H, δ 6·30 (s, 1H); C-5 OH, 13·52 (s, 1H); C-6 Ac, 2·68 (s, 3H); C-7 H, 8·08 (d, J = 8 Hz, 1H); C-8 H, 7·65 (d, J = 8 Hz, 1H). The mass spectrum gave the mol wt 232.

Fries reaction on 3-hydroxyjuglone leucoacetate. 3-Hydroxyjuglone (300 mg) furnished 1,3,4,5-tetraacetoxynaphthalene, m.p. 161–162°, white needles from EtOH (500 mg, 88%). The mass spectrum confirmed the mol wt of 360. NMR spectrum (acetone–d_6): C-1 OAc, δ 2·47 (s, 3H); C-3 OAc, 2·33 (s, 3H); C-4 OAc, 2·44 (s, 3H); C-5 OAc, 2·40 (s, 3H); C-2, C-6, C-7 and C-8 H's, 7·03–7·95 (m, 4H). The Fries reaction on 1,3,4,5-tetraacetoxynaphthalene (400 mg) furnished three products by TLC.

Band 1 was crystallized from $CHCl_3$–isooctane, m.p. 129–132°; UV spectrum; λ_{max} 283 and 415 nm. A tentative structure of 2-acetyl-3-hydroxyjuglone was adducted. Although the mass spectrum showed a probable molecular ion m/e 232, a large peak at 218 could not be rationalized. The compound was then rechromatographed by TLC using repeated elutions in CCl_4 over a 48 hr period. Two yellow compounds

were isolated. The slower band had a mol wt of 218 and was not identified. The faster band showed a mol wt of 218 and was not identified. The faster band showed a mol ion at *m/e* 232. UV spectrum: λ_{max} 281 and 405 nm.

Band 2 contained 3-hydroxyjugalone.

Band 3 was the major yellow band and was isolated and crystallized from $CHCl_3$–isooctane. Its UV spectrum had λ_{max} 237, 278 and 418 nm and was identical with the major product obtained by boiling 6-acetyljuglone in EtOH–HCl. The R_f values were also identical. The mass spectrum gave a mol wt of 232 and a structure of 6-acetyl-3-hydroxyjugalone was assigned.

2,3-*Dihydroxyjuglone.* Following the procedure of Garden and Thomson[9] the 100 MHz NMR spectrum of juglone methyl ether epoxide in $CDCl_3$ gave C-2 and C-3 H's, δ 3·975 (s); C-5 OMe, 3·95 (s); C-6, C-7 and C-8 H's, 7·21–7·74 (ABX multiplet). The 60 MHz NMR spectrum ($CDCl_3$) gave C-2 and C-3 H's, δ 3·98 (s, 2H); C-5 OMe, 3·95 (s, 3H); C-6, C-7 and C-8 H's, 7·21–7·72 (m, 3H). Upon shaking with D_2O no change in the NMR spectrum occurred.

Ac_2O and H_2SO_4 converted the epoxide into 2,3-diacetoxy-2,3-dihydro-5-methoxy-1,4-naphthoquinone. The NMR spectrum (100 MHz, $CDCl_3$) gave C-2 and C-3 H's, δ 5·84 (s); C-2 and C-3 OAc, 2·255 (s); C-5 OMe, 3·975 (s); C-6, C-7 and C-8 H's, 7·27–7·84 (ABX multiplet). The 60 MHz NMR spectrum (acetone–d_6) gave C-2 and C-3 H's, δ 6·00 (s, 2H); C-2 and C-3 OAc, 2·19 (s, 6H); C-5 OMe, 3·96 (s, 3H); C-6, C-7 and C-8 H's, 7·5–7·89 (m, 3H). Shaking with D_2O produced no change in the NMR spectrum.

Base hydrolysis followed by shaking with air for a few seconds easily gave 2,3-dihydroxy-5-methoxy-1,4-naphthoquinone. When $NaAlCl_4$ was used to demethylate the yield was quite low as compared with Thomson's report. However, the yield was comparable and a cleaner reaction resulted when 48% HBr soln was added and the mixture refluxed under N_2. 2,3-Dihydroxy-5-methoxy-1,4-naphthoquinone (100 mg) was placed in a flask with a condenser and flushed with N_2. Ten milliliter of 48% HBr solution was added and the mixture was refluxed under N_2 for 5 hr. The red soln became dark during the reaction. It was cooled and extracted with $CHCl_3$. The extract was dried and evaporated. The residue was chromatographed on a column using $CHCl_3$ as the eluant. The only major yellow band furnished pure 2,3-dihydroxyjuglone, 27 mg or 29% yield. The UV spectrum ($CHCl_3$) gave λ_{max} 239, 257, 295 and 418 nm. The UV spectrum (95% EtOH) gave λ_{max} 248, 275sh, 403 and 485sh nm. The pure compound began to sublime at ca. 200° and melted at 234° (dec). Thomson reported that the vis spectrum (95% EtOH) had λ_{max} 397 and 567 nm, m.p. 234° (dec).[21] The mass spectrum gave a parent peak at *m/e* 206 which was also the base peak.

Fries reaction on 2,3-*dihydroxyjuglone leucoacetate.* 2,3-Dihydroxyjuglone (100 mg) furnished 1,2,3,4,5-pentaacetoxynaphthalene (175 mg, 88%), crystallized from EtOH–H_2O (1 : 1) as gray prisms, m.p. 167–168°. The NMR spectrum (acetone–d_6) had signals for C-1 OAc, δ 2·45 (s, 3H); C-2 and C-3 OAc, 2·33 (s, 6H); C-4 OAc, 2·40 (s, 3H); C-5 OAc, 2·36 (s, 3H); C-6, C-7 and C-8 H's, 7·17–8·06 (m, 2·6H).

The leucoacetate (100 mg) on Fries reaction and usual work-up yielded essentially a single yellow band by column chromatography, 51 mg (87%), 6-acetyl-2,3-dihydroxyjuglone. It was further purified by TLC and the main slow moving band (ca. 95%) was crystallized as red needles from $CHCl_3$–isooctane, m.p. 201°. The NMR spectrum (acetone–d_6) had signals for C-6 Ac, δ 2·7 (s, 3H); C-7 H, 8·06 (d, J = 8 Hz, 1H); C-8 H, 7·62 (d, J = 8 Hz, 1H). The UV spectrum had λ_{max} 267, 297 sh and 410 nm.

6-Acetyl-2,3-dihydroxyjuglone (30 mg) was dissolved in 15 ml MeOH and $NaBH_4$ was added in small amounts with continuous stirring for 15–20 min. The reaction mixture was decomposed over ice–HCl and extracted with ether. The ethereal extract was shaken with NaOH aq for a few sec and the dark brown aqueous layer poured into ice–HCl. It was extracted with ether, dried, and the ether was evaporated. The residue was subjected to preparative TLC. The first band has been tentatively assigned the structure of 6-ethyl-3-hydroxyjuglone on the basis of relative R_f values and a UV spectrum of the impure sample. The second band contained 2,3-dihydroxy-6-ethyljuglone (12 mg, 41%) which was crystallized from $CHCl_3$–isooctane, m.p. 190–191° (dec). The 60 MHz NMR spectrum (acetone–d_6) showed signals for C-6 methylene, δ 2·73 (q, J = 7·5 Hz); C-6 $CH_2\underline{CH_3}$, 1·22 (t, J = 7·5 Hz); C-7 and C-8 H's, 7·54 (s). The 100 MHz NMR spectrum ($CDCl_3$): C-5 OH, δ 11·69 (s, 0·8H); C-6 methylene, 2·75 (q, J = 7·5 Hz, 2·4H); C-6 $CH_2\underline{CH_3}$, 1·26 (t, J = 7·5 Hz, 3·0H); C-7 H, 7·41 (d, J = 7·0 Hz, 1·1H); C-8 H, 7·58 (d, J = 7·5 Hz, 1·1H). The UV spectrum had λ_{max} 255, 300 and 428 nm. The mass spectrum confirmed the mol wt of 234.

The third band consistedof 8 mg starting material.

If the reaction time was increased, the yield of 2,3-dihydroxy-6-ethyljuglone decreased and the yield of 6-ethyl-3-hydroxyjuglone increased.

Methylation of 2,3-dihydroxy-6-ethyljuglone was achieved rapidly upon treatment with diazomethane in ether. After purification by TLC 2,3-dimethoxy-6-ethyljuglone was crystallized from $CHCl_3$-isooctane

as orange needles, m.p. 71°; UV spectrum: λ_{max} 251, 297 and 427 nm. The mass spectrum confirmed the mol. wt of 262.

Fries reaction on 2,3,7-trihydroxyjuglone leucoacetate. 2,3,7-Trihydroxyjuglone (30 mg) furnished 41 mg (64%) of 1,2,3,4,5,7-hexaacetoxynaphthalene, m.p. 225–228°, mol. wt (mass spec) 476.

The leucoacetate (35 mg) on Fries reaction, hydrolytic oxidation, and methylation with diazomethane yielded after TLC purification 6-acetyl-7-hydroxy-2,3-dimethoxyjuglone identical with the dimethyl ether of the natural product;[3] UV spectrum: λ_{max} 310, 371, 464 nm. Mol. wt. (mass spec), 292.

Fries reaction on 2,7-dihydroxyjuglone leucoacetate. By the previous procedure 2,7-dihydroxyjuglone (20 mg) was reductively acetylated to obtain 1,2,4,5,7-pentaacetoxynaphthalene (19 mg, 43%). The leucoacetate (15 mg) was dissolved in 5 ml Ac_2O and BF_3 was bubbled through the soln. The temp rose to 108°. The reaction soln was worked up as above and the residue analyzed.

Band 1 was 6-acetyl-2,7-dihydroxyjuglone (2 mg, 20%). It was identical with the natural pigment, designated $2B_2$, isolated from the spines of *Echinothrix diadema* Linn.[3] The mass spectrum confirmed a mol. wt. of 248.

Band 2 was a minor unidentified constituent.

Band 3 was also a minor compound which had an R_f value identical with that of 3-acetyl-2,7-dihydroxyjuglone, the natural pigment $6AM_{18}$ isolated from *E. diadema*.[3]

Relative R_f *values.* The following Table lists relative R_f values of juglone derivatives as observed TLC plates, prepared with dsg, and eluted with benzene.

Compound	Relative R_f values in benzene
3-Ethyl-2-hydroxyjuglone	1·01
Juglone	1·00
3-Acetyl-2-hydroxyjuglone	0·84
2,3-Dimethoxy-6-ethyljuglone	0·69
6-Acetyljuglone	0·65
2-Acetyl-3-hydroxyjuglone	0·63
6-Ethyl-2-hydroxyjuglone	0·58
2-Hydroxyjuglone	0·57
6-Acetyl-2,7-dihydroxyjuglone	0·43
6-Acetyl-1,5-dihydroxynaphthalene	0·42
5-Methoxy-1,4-naphthoquinone	0·34
3-Hydroxyjuglone	0·33
6-Acetyl-2-hydroxyjuglone	0·23
2,3-Dihydroxy-6-ethyljuglone	0·17
2,3-Dihydroxyjuglone	0·11
6-Acetyl-3-hydroxyjuglone	0·11
7-Hydroxyjuglone	0·10
2,3-Dihydroxy-5-methoxy-1,4-naphthoquinone	0·09
3-Acetyl-2,7-dihydroxyjuglone	0·06
6-Acetyl-2,3-dihydroxyjuglone	0·034

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