Conocurvone—Prototype of a New Class of Anti-HIV Active Compounds?**

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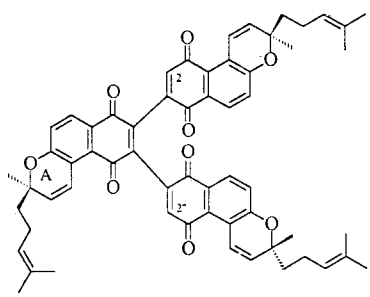
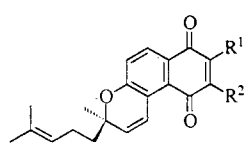
Workers at the National Cancer Institute recently achieved a remarkable success in their search for active plant metabolites. During routine screening, extracts of a shrub indigenous to Australia were noted for their exceptionally high, and, more importantly, selective anti-HIV activity in a variety of cellular in vitro tests. The substance responsible for this effect was the naphthoquinone conocurvone **1a**, which was isolated after an elaborate purification procedure in a yield of 22 mg per kg plant material (*Conospermum sp. Proteaceae*).[1]

As fluorescence tests with BCECF (2',7'-bis-2(2-carboxyethyl)-4(5)-carboxyfluorescein), the XTT–tetrazolium test, and a test with the intercalating dye DAPI (4',6-diamidino-2-phenylindole dihydrochloride) showed, the presence of conocurvone **1a** at a concentration of $EC_{50} \leq 0.02$ μM completely averted the death of HIV 1-infected human lymphoblastoid cells (CEM–SS). Measurements of viral reverse transcriptase, the viral P24 antigen, and the syncytium forming units (SFU) revealed that virus replication came to a standstill at the same time. Since conocurvone **1a** is cytotoxic and inhibits growth only at or above a concentration of 50 μM, the therapeutic index has the unusually high value for a virostatic of 2500. Whether this value also holds for other viruses, or is limited to HIV-1 viruses, was not reported.



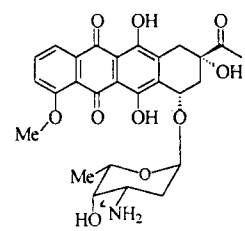
1a
1b: Hydrogen instead of ring A



2a: R^1 = OH, R^2 = H
2b: R^1 = R^2 = H
2c: R^1 = H, R^2 = *p*-$O_2CC_6H_4Br$
2d: R^1 = R^2 = SC_6H_5

Conocurvone **1a** is a deoxy-trimer of teretifolion B (**2a**), a compound that has been known for longer and was first isolated from *Conospermum teretifolium*. The fast atom bombardment (FAB) mass spectrum of the trimeric quinone revealed a molecular ion corresponding to the formula $C_{60}H_{56}O_{11}$, but the structure of **1a** was in the end only fully elucidated by synthesis. The reason for this was that atropoisomeric equilibria were formed and led to more or less complex ^1H NMR spectra that varied with solvent and with temperature. The compound therefore appeared to be a complex mixture. However, the synthetic product was identical to the natural material, even in its chiroptical parameters, thus confirming the structure and also the low rotation barrier around the quinone–quinone axis, a property which has also been found for other quinonoid-quinonoid-coupled oligomers.

Quinones constitute an extremely important group of natural products. They are one of the oldest known classes of compounds, have achieved importance as dyestuffs, and in addition possess many different biological activities: many quinones are active as antibiotics, are cytotoxic (like daunomycin (**3**), or are (weakly) antiviral. Even simple compounds such as plumbagin (**4a**) prove to be highly effective and selective as enzyme inhibitors. For other quinones the activity derives from interaction with physiological redox systems, or—at least for unsubstituted quinonoid double bonds—from the reaction with nucleophiles.[2]



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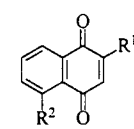

4a: R^1 = Me, R^2 = OH
4b: R^1 = H, R^2 = OH
4c: R^1 = OH, R^2 = H

In the case of conocurvone (**1a**) a physiological effect has once again been found for an oligomeric quinone, which is completely absent for the monomer **2a**. This must therefore be a new property that arises from the *oligomerization*, and should thus stimulate renewed interest in oligonaphthoquinone synthesis. A brief outline of the current state of knowledge about this class of substances is hence justified.

In all but a few cases (one of which is **1a**), the over seventy naturally occurring dimeric, trimeric, *cyclo*-trimeric, and higher oligomers are all constructed of identical units derived from juglone (**4b**, 5-hydroxynaphthoquinone). These units are linked together in various ways, symmetrically or asymmetrically with respect to the C–C framework. With the exception of crisamicin A, all these compounds have an oxygen atom in the *ortho* position to the linkage site.

The reason for this variety lies in the mechanism of formation of dimeric quinones, which may be regarded as a phenol oxidation. The synthesis starts from substituted naphthols, such as **5b**, and leads, depending on the initial substrate and the enzymes of the organism concerned, not only to the monomeric quinone but via binaphthols (e.g. vioxanthin (**5c**)) also to dimeric (e.g. xanthomegnin (**5d**)) or oligomeric quinones. It also yields the polymeric, black allomelanin, which gives ebony, for instance, its characteristic color. This pathway is confirmed by the presence—often in the same organism—of dimers with lower oxidation states, which are formal intermediates in diquinone

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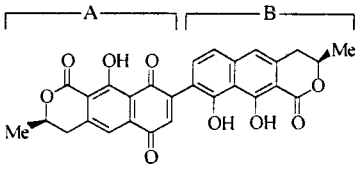
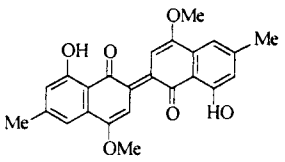
biosynthesis: examples are the monoquinone viomellein **5a** and the binaphthylidenedione (**6**, diosindigo A).



5a ≙ A–B
5b: only component B
5c: B instead of A in **5a**
5d: A instead of B in **5a**



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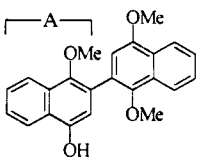
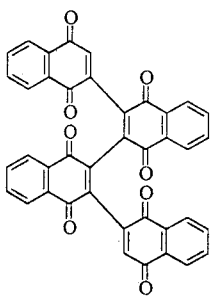
In nature, the oxidative dimerization of phenols is controlled by enzymes, as is demonstrated by the axial chirality of the 6,8′-coupled juglone derivative isodiospyrin. In synthesis, however, phenol oxidation only proceeds in high yields when the enzymatic reaction control is replaced by substituent control, that is, if all but one of the positions with high spin density in the radical (*ortho* and *para* positions) are blocked.

The synthetic usefulness of this principle is well documented by numerous examples, even under biomimetic conditions.[3] In this way, using a similar synthetic sequence by co-oxidation of **7a** and **7b**, we have obtained not only the dimers, but also quinones **8a** and **8b**. These are related to conocurvone **1a**, but their antiviral properties have not yet been studied.



7a
7b: only component A

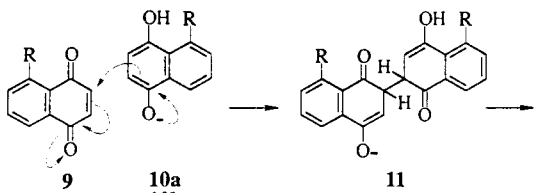


8a
8b: trimer instead of tetramer

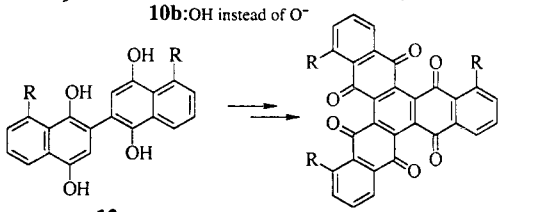
Unlike in the case of phenols, direct oxidative dimerization of quinones only takes place under drastic conditions and requires a hydroxy or amino group on the quinonoid double bond, as in Lawson **4c**, the dye in Arabian henna. Since the work of Pummerer (1937), however, it has been known that monomeric 1,4-quinones can be oligomerized much more easily under acidic or basic conditions.[5] In pyridine/ethanol, or by warming in glacial acetic acid, we have converted naphthoquinone, 1,4-anthraquinone, and numerous derivatives—though not benzoquinones—smoothly into dimers and *cyclo*-trimers. In this highly regioselective reaction, juglone (**4b**) and several of its derivatives afford exclusively the symmetrical 3,3′-linked dimers, several of which were already known as natural products.[6] It was only recently discovered that *o*-quinones may also be dimerized by this principle.[7]

According to Brockmann, this process is an autocatalytic one, in which traces of hydroquinone, which are always present, undergo a Michael addition to the excess of quinone to form a biaryltetrol (**12**),[8] an intermediate which also occurs during phenol oxidation. Dehydrogenation of this intermediate by the monomeric quinone yields the biaryldiquinone and further hydroquinone, until all the monomeric quinone has been transformed ($3\,\mathbf{9} + x\,\mathbf{10c} \rightarrow \mathbf{12c} + \mathbf{10b}$). When air is excluded, conversions of naphthoquinone reach 90%; when air is present, or when nitrobenzene is used as solvent, the yield of dimer can be increased even more through reoxidation of the hydroquinone.

Under suitable reaction conditions, the dimer that is initially formed can react further to yield trimers of type **8b**. It has not yet been possible to isolate these compounds, since they are always converted into the trimeric cyclic quinones **13a**. Indeed, **8b** is also converted into **13a** by phenol oxidation under weakly basic conditions. The stability of conocurvone **1a** is therefore due to the presence of hydroxy groups at position 2 of the monomer, which prevent cyclization. This cyclization via a symmetrical dimer of type **12b** explains why none of the known hydroxylated *cyclo*-trimers (e.g. *cyclo*-trijuglone **13b**) display C_3-symmetry with respect to the *peri*-hydroxy groups.



9 **10a** **11**
10b: OH instead of O⁻



12a: R = H
12b: R = OH
12c: R = H, quinone instead of hydroquinone

13a: R = H
13b: R = OH

The synthesis of conocurvone (**1a**) also makes use of the well established principle of phenol/quinone addition, even though the term was inspired by the reaction of quinones with hydroxy-2*H*-1-benzopyran-2-ones, a reaction that was discovered later but proceeds in a similar manner. Brief warming of two equivalents of teretifolion B (**2a**) with naphthoquinone in glacial acetic acid afforded the predicted **1b** in 9% yield. For the synthesis of conocurvone (**1a**), 2-deoxyteretifolione (**2b**) was warmed with two equivalents of **2a** in pyridine, analogously to the synthesis of 2,2′-binaphthyldiquinone. All the spectroscopic and pharmacological properties of the synthetic material agreed with those of the natural product.

The main difficulty in the synthesis of **1a** lay in the deoxygenation of **2a** to **2b**. This was eventually carried out by transforming

the *p*-bromobenzoate **2c** to **2d** with thiophenol, with subsequent catalytic reduction with Raney nickel.

Nothing is yet known about the site of action or structure–function relationships for derivatives of **1a** (as, for example, **1b**), and any discussion is therefore very speculative in nature. However, it is conceivable that **1a** assumes a helical conformation which winds into the groove of the DNA strand. Similar conformations are also expected for **8a**, and in particular for **8b** and higher oligomers. As we have shown, partial reduction of these compounds leads to deep blue, intramolecular quinhydrones, which are also stable in solution and can be shown by molecular modeling to be stabilized in a helical conformation. For **1a**, additional interactions may be expected between the quinonoid hydroxy groups and petides, like those that play a role in coloring hair and skin with henna (**4c**). It will be fascinating to see whether these hypotheses are confirmed.

German version: *Angew. Chem.* **1994**, 106, 438.

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