CHEMISTRY OF HYPERFORIN
6. GENERAL CHEMICAL CHARACTERISTICS

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[Translated by Google Translate.]

The reactions of acylation, C- and O-alkylation, catalytic hydrogenation, reduction with lithium aluminum hydride, and the oxidation of permanganate with the antibiotic hyperforin and its derivatives were studied. It is established that hyperforin is a bicyclic enolized tetraketone and has four side chains, each of which terminates in an isobutenyl group.

In 1971, we described a new antibacterial antibiotic from St. John's wort (Hypericum perforatum L.), called hyperforin [2]. This and the following reports are devoted to a detailed description of the studies, as a result of which the structure and configuration of this antibiotic, which were published in preliminary reports, were established [1, 3, 4].

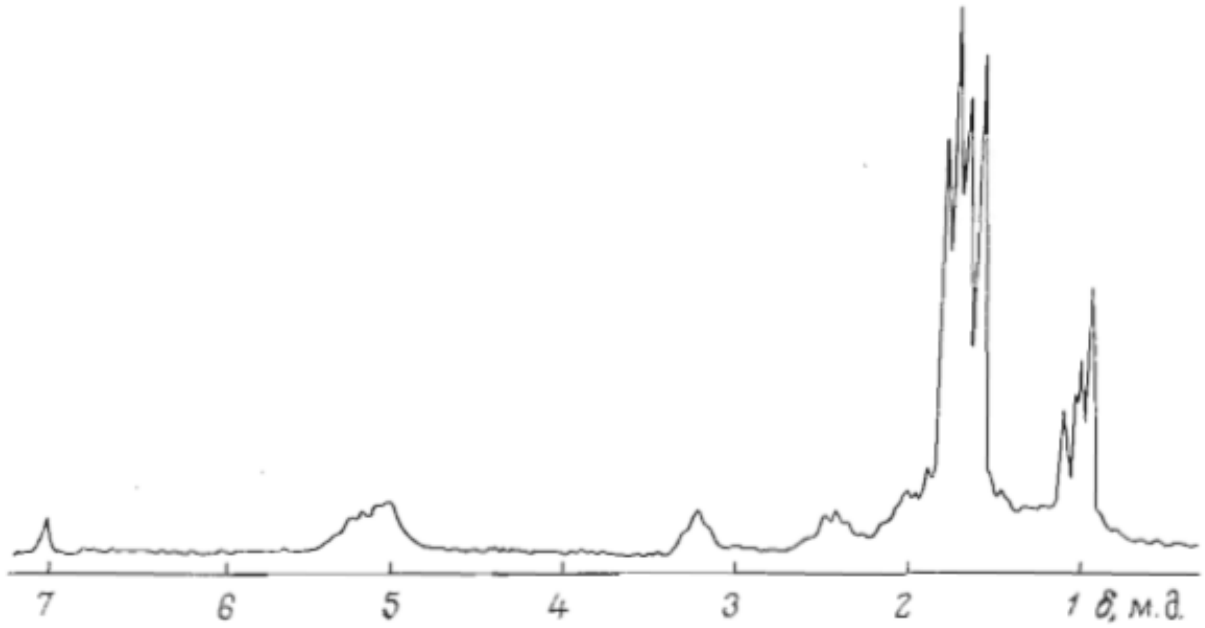
Hyperforin (1) was isolated from St. John's wort acetone extract by adsorption chromatography on silica gel with gradient gradient glutinization with a mixture of petroleum ether - benzene in the presence of ionol as an antioxidant. Further purification was carried out by converting hyperforin to crystalline 3,5-dinitrobenzoate (II), from which a pure antibiotic was obtained by the action of 0.1 N alcohol alkali; there was no loss of antimicrobial activity.

UV spectra of hyperforin

As a result of elemental analysis and mass-spectrometric determination of molecular weight, it was found that hyperforin has empirical formula $C_{35}H_{52}O_4$ (MW 536). In the IR spectrum of the antibiotic there are absorption bands of OH groups, C=O (conjugate and nonconjugated) and C = C (conjugate and nonconjugated). UV hyperforin spectrum (table) depends on the concentration and pH of the solution, which indicates the ionic nature of its chromophore group, which, according to spectrophotometric titration, has a pK of 4.8. Judging by the NMR spectrum (see Figure), the antibiotic contains 11 C-methyl groups, one of which is found in the quaternary, two in the tertiary, and eight at olefinic C-atoms. In addition, it has four olefin protons (δ 4.8–5.3 ppm) and one H atom (6–7 ppm), which is easily exchanged for deuterium in D_2O and obviously must belong to enol hydroxyl.

To determine the gains of the chromophore grouping of hyperforin, the spectral properties of its derivatives were studied. So, it was found that upon acetylation with acetic anhydride in pyridine, an O-mono-acylated derivative of hyperforin (IV) is formed, with a maximum UV absorption (250 nm) strongly shifted to the short-wavelength region. Compared with the initial antibiotic, 4-methylation of hyperforin with diazomethane leads to an O-methyl derivative (III)

(λ_{max} 270 nm), the UV spectrum of which does not depend on the concentration of the pH of the solution. As a result of alkylation of the antibiotic with methyl iodide and sodium hydride in dimethyl sulfoxide forms its C-monomethyl derivative (V). The UV spectrum of this compound indicates the presence of a nonconjugated ketone chromophore (λ_{max} 294, 303, ε 8142, 138), and judging by its NMR spectrum, a new methyl group (singlet at 1.54 ppm) is linked to a quaternary carbon atom. Thus, the characteristic changes in the UV spectrum of hyperforin upon O-acylation and C-alkylation, the dependence of the absorption maximum and extinction on the concentration and pH of the solution, as well as the acid properties of the antibiotic, indicate that its ionizable chromophore group has the structure of the enolized α-substituted β-diketone and that with C-methylation, the incoming group occupies a position between the carbonyls, preventing their enolization.



NMR spectrum of hyperforin (I) in CCl_4 at 100 MHz.

When hyperforin is reduced in excess of $LiAlH_4$, tetrahydro derivative (IX) forms. The UV spectrum of this compound is similar to the spectrum of hyperforin, and therefore, it retains an enolized diketone moiety. At the same time, unlike hyperforin, this tetrahydroderivative has three active H-atoms exchanging from D_2O to deuterium (mass spectrometric determined). Since one H_{act} is contained in the enolized beta-diketone group, the other two must belong to the hydroxyls formed as a result of the reduction of two ketone antibiotic groups. Under the action of acetic anhydride on tetrahydrohyperforin in pyridine, monoacetate (X) was obtained, in the IR spectrum of which there is a band of 1775 cm^{-1} characteristic of acyl derivatives of enols. During its subsequent processing, the ionogenic nature of its chromophore group, which, according to spectrophotometric titration, has a pK_a of 4.8. Judging by the NMR spectrum (see figure), the antibiotic contains 11 C-methyl groups, one of which is found at the quaternary, two at tertiary and eight at olefinic C-atoms. In addition, it has four olefin protons (δ 4.8-5.3

ppm) and one H atom (δ ~7 ppm), which is easily exchanged for deuterium in D_2O and, obviously, should belong to the enol hydroxy.

To determine the nature of the chromophore grouping of hyperforin, the spectral properties of its derivatives were studied. Thus, it was found that upon acetylation with acetic anhydride in pyridine, the O-monoacetyl derivative of hyperforin (IV), in which the maximum UV absorption (250 nm) is strongly shifted to the shortwave region compared to the original antibiotic. Hyperforin methylation with diazomethane leads to an O-methyl derivative (III) (λ_{max} 270 nm), the UV spectrum of which does not depend on the concentration and pH of the solution. As a result of alkylation of the antibiotic with methyl iodide and sodium hydride in dimethyl sulfoxide. its C-monomethyl derivative (V) forms. The UV spectrum of this compound indicates the presence of a nonconjugated ketone chromophore (λ_{max} 294, 303 nm, ε 142, 138), and judging by its NMR spectrum, a new methyl group (singlet at 1.54 ppm) linked to the quaternary carbon atom. Thus, the characteristic changes in the UV spectrum of hyperforin during O-acylation and C-alkylation, the dependence of the position of the absorption maximum and extinction on the concentration and pH of the solution, as well as the acidic properties of the antibiotic, indicate that its ionizable chromophore group has the structure of the enolized α-substituted β-diketone and that with C-methylation, the incoming group occupies a position between the carbonyls, preventing their enolization.

When hyperforin is reduced in excess of $LiAlH_4$, a tetrahydro derivative (IX) is formed. The UV spectrum of this compound is similar to the spectrum of hyperforin, and, therefore, it retains the enolized diketone group of the antibiotic. At the same time, unlike hyperforin, this tetrahydro derivative has three active H atoms exchanging with D_2O for deuterium (mass-spectrometrically determined). Since one H_{act} is contained in the enolized β-diketone group, the other two must belong to the hydroxyls resulting from the reduction of two ketone antibiotic groups. Under the action of tetrahydroperforin with acetic anhydride in pyridine, monoacetate (X) was obtained, in the IR spectrum of which there is a band of 1775 cm^{-1} characteristic of acyl derivatives of enols. During its subsequent treatment with phosgene and dimethylamine in order to obtain bis-urethane, a cyclic carbonate (XI) was formed. The presence of the C=O absorption band in its IR spectrum at 1765 cm^{-1} indicates that the carbonate cycle is six-membered, from which, in turn, follows the 1,3-location of alcohol groups in enol acetate and the corresponding ketone groups in hyperforin. Therefore, the antibiotic contains two β-diketone groups: one conjugated, enolized, and one nonenolized.

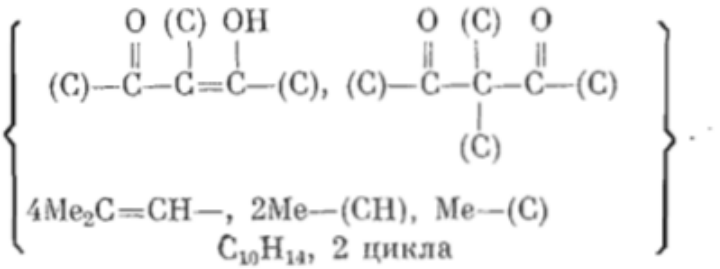
When hydrogenated over a Pt or Pd catalyst, hyperforin absorbs 4 moles of hydrogen rapidly, forming an octahydro derivative (XII), which, judging by the UV spectrum, retains the antibiotic beta-diketone chromophore. In the NMR spectrum of octahydrohyperforin, there are no signals: the antibiotic has no olefinic protons, and the signals of the 8 methyl groups, located at 1.51.8 ppm, are shifted towards a strong field. This shows that the antibiotic contains four C=C bonds.

Like hyperforin, C-methylhyperforin, upon catalytic hydrogenation with Pd, easily absorbs 4 mol of hydrogen, forming the corresponding octahydro derivative (VIII). This substance turned out to be resistant to strong acids and oxidizing agents such as KMnO4. Using this circumstance, we subjected C-methyl-octahydrohyperforin to acid-catalyzed deuteration with D_2SO_4 in a D_2O–dioxane solution and found (mass spectrometrically) that even under these forced conditions only one hydrogen atom is replaced with deuterium, i.e. its molecule contains only one CH group in the α-position of the ketone carbonyl.

On the other hand, taking into account the inertness of C-methyloctahydrohyperforin to the action of $KMnO_4$ (and, consequently, the stability of the main part of the carbon skeleton of hyperforin), we undertook directed degradation of the antibiotic by olefinic bonds. During the oxidation of C-methylhyperforin with a mixture of $KMnO_4$ + $NaIO_4$, it was found that acetone was formed as the only volatile product, the amount of which exceeds 3 mol (determined iodometrically and in the form of 2,4-dinitrophenylhydrazone). From non-volatile oxidation products, acid (VI) was isolated, which was called methyl hyperforin, which, under the action of diazomethane, gave tetramethyl ether (VII). It follows that in C-methylhyperforin (and, consequently, in the antibiotic itself) there are 4 isobutenyl groups — $CH=CMe_2$.

The empirical formula of hyperforin indicates the presence of 10 increments of double bonds and cycles. As shown above, four of them should be assigned to isobutenyl groups, and another four to carbonyl groups. Obviously, the remaining two increments are due to the presence of two carbon rings. Thus, hyperforin is a bicyclic enolized tetraketone and has one tertiary and two secondary C-methyl groups, as well as four side targets that end with isobutenyl residues:

$C_{35}H_{52}O_4$ = гиперфорин



The detailed formula described above summarizes the information about the structure of the antibiotic obtained at this stage of its study. For convenience, the complete structural formulas of the compounds under discussion, which were ultimately established by the entire series of studies, are also given below.

Experimental part. [not translated]

Literature.

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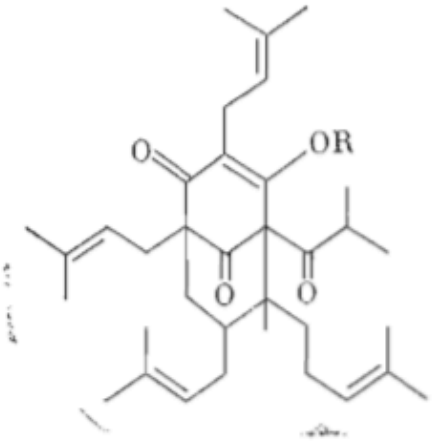
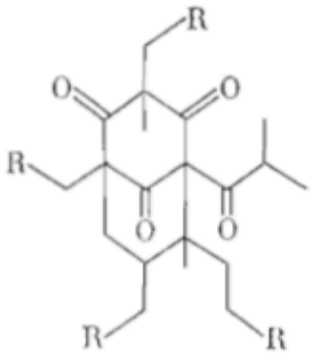
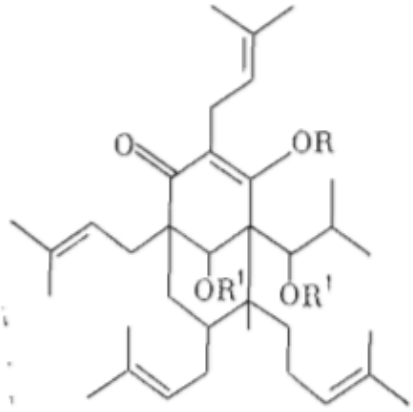
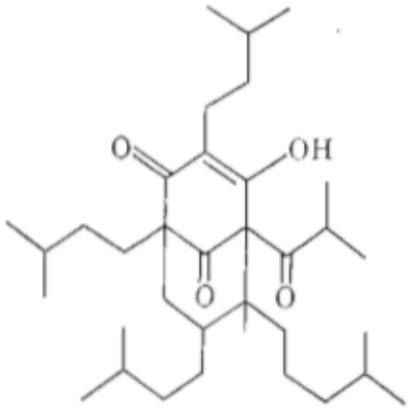
2. Gurevich A. I., Dobrynin V. N., Kolosov M. N., Popravko S. A., Ryabova I. D., Chernov B. N., Derbentseva N. A., Aizenman V. E., Garagulya A. D. (1971) *Antibiotics*, 510–512.
3. Bystrov N. S., Chernov B. K., Dobrynin B. N., Kolosov M. N. (1975) *Tetrahedron Lett.,* 2791–2794.
4. Bystrov N.S., Punta Sh. R., Dobrynin V.N., Kolosov M.N., Chernov B.N., Chervin I.I., Yakovlev G. I., Aizenman V. E., Derbentsova N.A. (1976) *Dokl. USSR Academy of Sciences*, *226*, 88–90.





(I) R = H
(II) R = $COC_6H_3(NO_2)_2$-3,5
(III) R = Me
(IV) R = Ac
(V) R = CH=CMe_2
(VI) R = CO_2H
(VII) R = CO_2Me
(VIII) R = CH_2CHMe_2





(IX) R = R' = H
(X) R = Ac, R' = H
(XI) R = Ac, R' + R' = >CO

(XII)