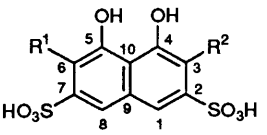
Biological Activity, Reactivity, and Use of Chromotropic Acid and its Derivatives

Jan Duda
Institute of General Food Chemistry, Technical University of Łódź, Łódź, Poland

1 Introduction

Chromotropic acid is the customary name of 4,5-dihydroxy-2,7-naphthalenedisulfonic acid (1a). This compound was synthesized last century and was originally used as a chrome dye. In the dyestuff industry, however, its azo derivatives (1d) and (1e) are now of greater importance. A profusion of these monoazo and bisazo dyes exists, covering a full colour palette. In recent years it has been found that derivatives of naphthalenesulfonic acids show some activity against viruses, including HIV.



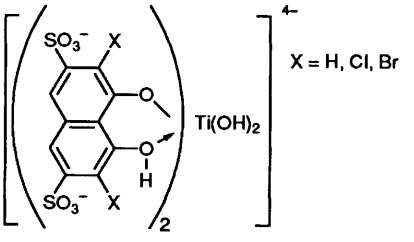
1a; $R^1 = R^2 = H$
1b; $R^1 = R^2 = Cl$
1c; $R^1 = R^2 = Br$
1d; $R^1 = H$, $R^2 = -N{=}N-Ar$
1e; $R^1 = R^2 = -N{=}N-Ar$

Chromotropic acid (H_2Chr) contains moieties which significantly affect its properties and determine its numerous applications. The peri-system of two hydroxyl groups determines the complex-forming properties of this compound and, on the other hand, together with the naphthalene ring makes it possible to create oxidized forms or naphthalene–quinone systems. Ring oxidizability can be affected by substituents in positions 3 and 6, *e.g.* halogen substituents (1b, 1c). Sulfonic groups (or their sodium salts) confer good water solubility on chromotropic acid and its derivatives.

This review will discuss the properties and numerous applications of chromotropic acid and its derivatives.

2 Complexing Properties

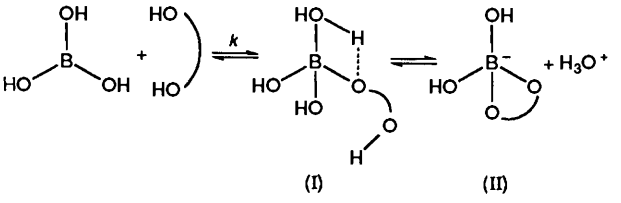
By the beginning of this century it was known that chromotropic acid formed complexes with metal ions – the first discovered being the Fe^{III} complexes. In 1952, the complexation of chromotropic acid and Ti^{IV} was discovered and put to use in the determination of Ti^{IV} ions. Over the following years many studies were published on the identification, composition, structure, and use of metal complexes with H_2Chr in chemical analysis. H_2Chr forms complexes with the following metal ions: Ca^{II}, Sr^{II}, Ba^{II}, Be^{II}, Al^{III}, Ga^{II}, In^{II}, Cr^{III}, Mn^{II}, Cu^{II}, Sn^{II}, U^{VI}, Mo^{IV}, Th^{IV}, Zr^{IV}, Ti^{III}, and lanthanide ions. An increase in chromotropic acid selectivity for Ti^{IV} ions can be obtained in the pH range 1 to 2. The determination of Ti^{IV}, with H_2Chr is an example of the most frequent use for H_2Chr, *viz.*, as a complexing agent. In attempts to improve the complexing reagent stability in solutions, halogen derivatives of chromotropic acid such as H_2ChrCl_2 (1b) and H_2ChrBr_2 (1c) have been synthesized.[1,2] The diiodic derivative H_2ChrI_2 has not as yet been obtained as a solid compound, but evidence for its formation in solutions has been found from examining the UV spectra of iodinated H_2Chr solutions. An investigation of the effect of substituents (Cl, Br) in the H_2Chr molecule on the stability of complexes (2) of H_2Chr, H_2ChrCl_2, and H_2ChrBr_2 with Ti^{IV} ions, has found that the stability of these complexes decreases in the order H_2Chr, H_2ChrCl_2, H_2ChrBr_2. The sensitivity of the determination of Ti^{IV} ions increases in the same direction, while the pH of the complexation process shifts towards more acidic solutions, *i.e.* more convenient reaction conditions from the point of view of limiting the hydrolysis of complexing metal ions as well as eliminating the influence of interfering ions.



X = H, Cl, Br

(2)

The complexation reaction of H_2Chr[6] or H_2ChrBr_2[7] with boric acid, H_3BO_3 has an interesting mechanism. The complex formation brings about changes in the coordination number of boron and its structure (Scheme 1). The kinetics of this reaction

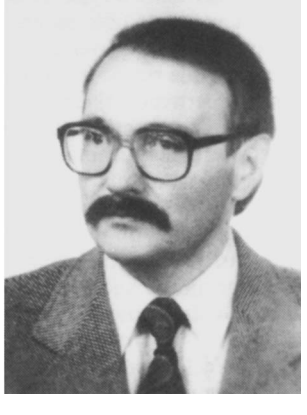


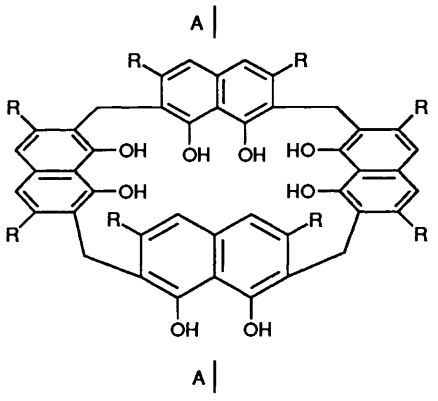
(I) (II)

Scheme 1

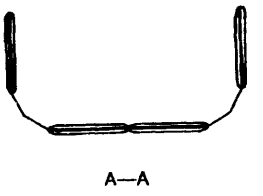
were investigated using a high-presssure stopped flow apparatus with spectrophotometric detection, which was utilized for the first time to that aim. In the first stage (I) bond formation with one hydroxyl group of the ligand results in a tetrahedral intermediate complex. The coordination of boron atom is thus changed at this stage from 3 (sp^2) to 4 (sp^3). In the next stage (II), another bond is formed with the oxygen atom of the second hydroxyl group resulting in a chelate ring, with dissociation of a water molecule. Thermodynamic and kinetic parameters for stage (I), which determines the reaction rate, have been found.[6]

Jan Duda was born in 1947 in Łódź, Poland. He graduated from the Technical University of Łódź with the M.Sc. and Ph.D. degrees in chemistry. His Ph.D. thesis concerned complexes of Ti^{IV}, Zr^{IV}, and Hf^{IV} with derivatives of chromotropic acid. He now lectures on analytical, inorganic, and instrumental chemistry at the Faculty of Food Chemistry and Biotechnology, Technical University of Łódź, and his research interests are in the fields of comprehensive coordination and analytical chemistry.

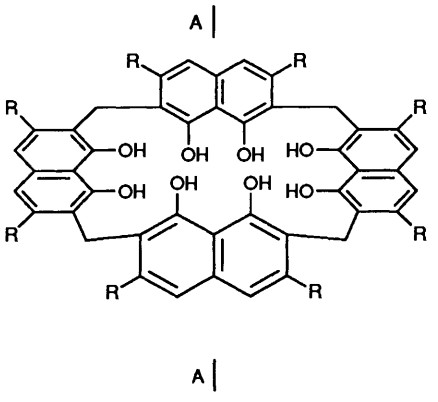




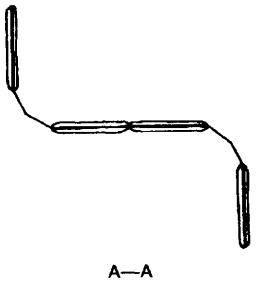
(4a, boat)



R = SO_3Na



(4b, chair)

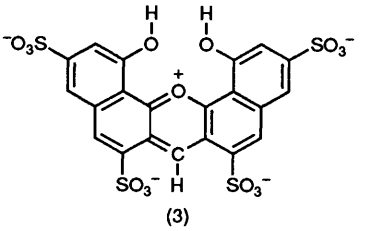


Beryllium is extremely toxic and an important achievement has been in the development of a method using H_2Chr for the determination of beryllium ions Chromotropic acid can neutralize the toxicity of beryllium(II), binding it into very stable complexes, and the stability constants of these complexes were determined utilizing an (atypical) ^{9}Be-NMR spectroscopic method [8] Owing to the slow exchange between the free ligand and the ligands combined into 1 1 and 1 2 complexes, it was possible to observe separate signals of $[Be(H_2O)_4]^{2+}$ and beryllium(II) in complexes with H_2Chr The signal intensities showed directly the concentrations of appropriate species, which enabled the stability constants to be calculated

As a result of all the complexes of chromotropic acid and its halogen derivatives being very soluble in water and aqueous solutions, there are only few papers devoted to the preparation of these complexes in the solid state In the literature one can find only the characteristic IR spectra of solid H_2ChrCl_2 complexes with Ti^{IV} ion[9] and H_2Chr complexes with ions for the following Cr^{III}, Fe^{III}, Co^{II}, Ni^{II}, Cu^{II}, Zn^{II}, V^{IV}, and Mn^{II}, [10] Measurements of the conductivity of solid chromotropic acid[11] with added of Zr^{IV}, or Ce^{III}, ions have been made in an attempt to examine the mechanism of charge-transfer by these complexes as a preliminary to their use in electrode reactions

3 Reactions with Formaldehyde

The colour reaction of chromotropic acid with formaldehyde has been used in chemical analysis since 1937 This is a characteristic reaction not only of formaldehyde but also of other compounds containing an aldehyde group Many other compounds can therefore be determined by this reaction following their conversion into an appropriate form, *e g* methanol and glycol after oxidation, formic acid after reduction The structure of the coloured compound (λ = 580 nm), formed in concentrated H_2SO_4 (96%), was confirmed in 1989 by Georghiou[12] using a ^{13}C-NMR method The structure of the compound is illustrated in (3)



(3)

A completely different compound has been obtained[13] from the reaction between H_2Chr and formaldehyde taken in the proportion 1 5 The resultant cyclic tetramer named cyclotetrachromotropylene is a representative of water-soluble macrocyclic compounds which can serve as model compounds for biological systems In an alkaline medium, the possible formation of intramolecular hydrogen bonding between adjacent O^- anions and $-OH$ groups leads to a boat structure rather than to a chair structure These conformers are presented schematically in formulae (4a) and (4b) Such a boat conformation can better play the part of host to guest cations This has been shown[13] with amines and protonated amines Depending on the size of the amine, this molecule can penetrate partly or completely into the cavity of the host from the face bearing the sulfonic acid groups Undoubtedly, this compound will also show selectivity in complexation processes with metal ions This cyclic tetramer has been found to be active against viral infection and its use has been patented [14] Its suitability as anticoagulant has also been confirmed [15]

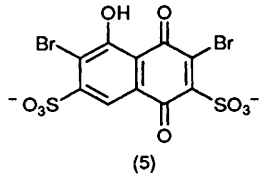
4 Oxidation Reactions of H_2Chr and its Derivatives

An aqueous solution of the disodium salt of H_2Chr, colourless when prepared, rapidly becomes yellow and then spontaneously becomes brown owing to gradual oxidation of the reagent Halogen derivatives such as H_2ChrCl_2 and H_2ChrBr_2 show higher stability to oxidation than does H_2Chr, H_2ChrCl_2 is about 400 times more stable than H_2Chr [1] To overcome this in all the applications of these compounds therefore, fresh solutions should be prepared just before use and stored, if necessary, in the dark Some metal ions show a catalytic effect on the oxidation of H_2Chr This, however, has also found positive applications

The Bielousov–Zhabotinskii reaction (BZR) has been found to occur with the participation of chromotropic acid [16] The 'classic' BZR is an oscillation process of oxidation of various organic compounds with a bromate solution ($KBrO_3$) in a strongly acidic medium in the presence of various metal-ion catalysts It was soon found that the reaction could proceed without catalysts, oscillations were also observed in a system of catalyst and bromate, without the participation of an organic compound So, the reasons for the oscillation lie in the reduction of bromate and appearance of reactive intermediate products Uncatalysed bromate oscillators (UBO) have been found in a system containing H_2Chr and potassium bromate [16] One could observe simultaneously potential oscillations on the platinum electrode and oscillations of the Br ion concentration on the bromo-selective electrode A five-stage scheme has been proposed for this type of oscillator, including, among other things, a bromide ion with autocatalytic effects Supposed products of oxidation of chromotropic acid have been identified by means of UV/VIS spectra as *o*-quinone derivatives of H_2Chr

More often the reactions between H_2Chr and $KBrO_3$, including the catalytic effect of metal ions, mainly vanadium(v), have been studied In these cases the concentrations of H_2Chr and bromate were lower by two or three orders of magnitude and the system was buffered to a pH of about 3 8, hence oscillation effects were neither observed nor considered This reaction has found its analytical application in the determination of vanadium ions in solution, and has been subjected to detailed investigations concerning the conditions of the reaction and the oxidized forms of H_2Chr created in this system [17 20] These studies have been extended by including halogen derivatives H_2ChrCl_2 [18 20] and H_2ChrBr_2 [19 20] It has been shown that the mechanism of oxidation of chromotropic acid is different from that of its halogen derivatives The oxidation of H_2Chr results in the desulfonation of its molecule and formation of polymeric *para*-naphthoquinone derivatives, which have not been separated as yet In the case of H_2ChrBr_2, a mixture of three oxidation products has been separated by chromatography Two of the oxidation products have been obtained in the solid state and identified by ^{1}H-NMR [20] The main product is the *para*-naphthalenequinone derivative (5) which amounts to about 45%, and this is further oxidized to compound (6) At the same time *ortho*-naphthalenequinone derivative is formed (7) It has been shown[19] that the oxidation process of H_2ChrBr_2 with $KBrO_3$ in the presence of vanadium(v) as catalyst makes it possible to determine vanadium down to a level of 1 ng/ml This is a five-fold improvement in sensitivity compared to the use of H_2Chr (detection limit, 5 ng/ml)

Redox reactions in the H_2Chr system have been utilized in the investigation of 8-hydroxy-1,2-naphthoquinone-3,6-disulfonic



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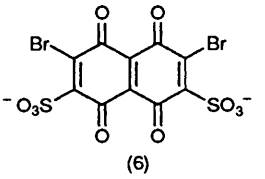

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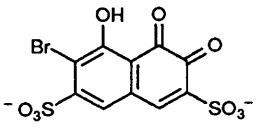

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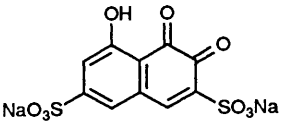

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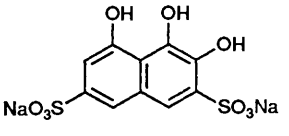


(9)

acid (8) Its reduced form 8-hydroxy-1,2-naphthohydroquinone-3,6-disulfonic acid (9) is a hydroxy derivative of chromotropic acid The oxidized form of this compound (8) has been found to show specific adsorption on the mercury dropping electrode [21] In further studies on the redox pair [compounds (8) and (9)] in the electrochemical reduction of oxygen to hydrogen peroxide,[22] compound (8) has been proposed as a mediator in the preparation of aqueous solutions of H_2O_2 [23] This redox pair can be also used in photoelectrochemical cells [22] This opens a new future field for the utilization of chromotropic acid and its derivatives

5 Biological Activity

Over 20 years ago[24] it was discovered that chromotropic acid showed some anti-viral effects (against the influenza virus among others), being effective both *in vitro* and *in vivo* Subsequently, the effects of simple and ternary complexes of Cu^{II}, Ni^{II}, and Zn^{II} with chromotropic acid on some bacteria and fungi have been investigated [25] It has been found that ternary complexes show higher activities than simple complexes and ligands separately

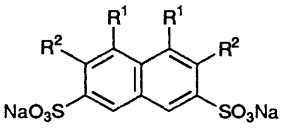
Particular attention has been paid to chromotropic acid and its derivatives as a result of the activity recently found (1992) in derivatives of sulfonic acids[26] against the human immunodeficiency virus (HIV)

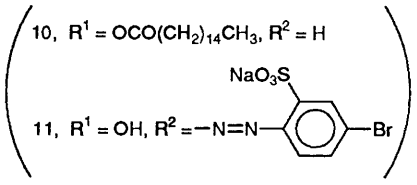
Among the viruses which cause AIDS, HIV-1 and HIV-2 have been distinguished These types differ, amongst other things, in the structure of their reverse transcriptase (RT), an enzyme which is important for viral replication Inhibition of RT is one of three postulated effects of chromotropic acid on HIV A second is the binding of virus envelope protein to its receptor on human T-lymphocytes A third is an action on the proteinase which participates in the virus replication process Some derivatives of chromotropic acid have been shown to react according the first and the third mechanisms

Of the 27 sulfonic acid derivatives studied[27] as potential RT inhibitors of HIV-1 and HIV-2, the highest activity has been shown by dipalmitoyl derivatives of chromotropic acid (10) Whilst chromotropic acid itself shows a higher inhibitary effect than most of the compounds examined, the activity of derivative (10) is almost 100 times higher The concentrations of compound (10) (in μM) bringing about 50% inhibition of HIV-1 and HIV-2 were IC_{50} = 2 4 and IC_{50} = 0 86, respectively These are lower concentrations than those of bis naphthalenetrisulfonic acid, suramine, previously used as an antitripanosomal agent, the inhibitary effects of which[26] have brought about an intensive search for anti-RT activity within this class of compounds Based on past investigations, one can postulate that the negatively charged sulfonic groups $-SO_3^-$ interact with positively charged sites on the enzyme An additional role may be played by the metal ion chelating properties of the derivatives It has been found that the optimum HIV-1 RT activity requires the presence of Mg^{2+} ions The binding of metal ions by chromotropic acid into stable chelate complexes should increase the activity of the compounds towards RT inhibition However, the

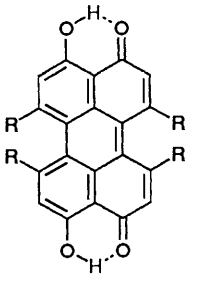
essential role of the lipophilic palmitoyl functionality in the high anti-RT activity of compound (10) has yet to be elucidated

The third mechanism (on proteinase) mentioned above is associated with the hypothesis that the 'ends' of the negatively charged inhibitor might form ionic bonds with arginine located at either ends of the substrate-binding groove of the enzyme Of the 32 derivatives[28] containing negatively charged groups separated by distances matching appropriate sites on HIV-1 proteinase, the bisazoderivative of chromotropic acid, bromosulfonazo III (11), has shown one of the highest inhibitions of synthetic HIV-1 proteinase activity (IC_{50} = 4)





Antitumour and protein kinase C inhibiting activity has been found in a substance isolated from the mycelium of the Chinese bamboo fungus (*Shiraia bambusicola*) A compound with an analogous carbon skeleton has been synthesized[29] from chromotropic acid by heating it with anhydrous aluminium chloride and sodium chloride The resulting compound (12) can exist as the acid (12a) or tetrasodium salt (12b) Of 27 similar compounds, tetrasodium 1,6,7,12-tetrasulfo-4,9-dihydroxy-3,6-perylenequinone (12b) has shown the highest activity against HSV-1 and HSV-2 viruses



12, a, R = SO_3H
b, R = SO_3Na

6 Conclusions

The reactions of chromotropic acid and its derivatives described above do not exhaust all the possible applications of these compounds For example, the use of H_2Chr in analytical chemistry is considerably wider than detailed and besides the above mentioned cations includes the determination of ions of such elements as Se^{IV}, and Te^{IV}, anions such as NO_2^-, NO_3^-, and ClO_3^-, as well as H_2O_2, and glucose in blood

The oxidizability of H_2Chr has been utilized in industry for the manufacture of photographic coatings, xerographic paper, for galvanic polishing of steel surfaces, and anodic coating of aluminium alloy surfaces One should expect new applications of chromotropic acid and its derivatives in reactions associated with electron exchange (photocells) Certainly, intensive studies will be continued on the biological activity of chromotropic acid derivatives, to utilize them in the struggle against AIDS

7 References

1 V I Kuznetsov and N N Basargin, *Zh Anal Khim*, 1961, **16**, 573
2 J Maslowska and J Duda, *Zesz Nauk Politech Lodz Chem*, 1974, **29**, 53
3 J Maslowska and J Duda, *Chem Anal (Warsaw)*, 1978, **23**, 805
4 J Duda, Ph D Thesis, Technical University of Lodz, 1977
5 J Maslowska and J Duda, *Acta Univ Lodz Zesz Nauk Univ Lodz Nauk Mat-Przyrod Ser II*, 1978, **24**, 59
6 K Ishikara Y Mouri, S Funahashi, and T Motoharu, *Inorg Chem* 1991 **30** 2356
7 M Bartusek, L Brchan, and L Havelkova, *Spisy prirodoved fak Univ J E Purkyne*, 1969, **499**, 19
8 D F Evans and C Y Wong, *J Chem Soc Dalton Trans*, 1992, 2009
9 J Duda and J Masowska, *Rocz Chem*, 1973, **47**, 899
10 N T Abdal-Ghani, Y M Issa, M A Khaled, and M H El-Kottamy *Thermochim Acta*, 1988, **125**, 165
11 E W Abd and G Mohamed, *Acta Chim Hung*, 1990, **127**, 51
12 P E Georghiou and C K Ho, *Can J Chem*, 1989, **67**, 871
13 B L Poh and C S Lim, *Tetrahedron*, 1990, **46**, 3651
14 K M Hwang, Y M Qi, and S Y Liu, *Pat PCT Int Appl WO*, **92**, 12709, 1992
15 K M Hwang, Y M Qi, S Y Liu, T C Lee, W Choy, and J Chen, *Pat CPT Int Appl WO*, **92**, 12708, 1992
16 L Kunert and H J Krug, *J Phys Chem*, 1990, **94**, 678
17 T Yamane, T Suzuki, and T Mukoyama, *Anal Chim Acta*, 1974, **70**, 77
18 A T Pilipenko, E R Falendysh, V G Safronova, and N F Falendysh, *Zh Anal Khim*, 1983, **38**, 1197
19 J Duda, *Pol J Chem*, 1991, **65**, 67
20 J Duda, *Chem Anal (Warsaw)*, 1993, **38**, 405
21 J Duda, *J Electroanal Chem*, 1991, **313**, 355
22 J Duda, *Pol J Chem*, 1991, **65**, 1701
23 J Duda and J Maslowska, *Pat PL* 161107, 1993
24 S Akerfeldt, G Westin, and T Janson, *Med Chem*, 1971, **14**, 596
25 R C Sharma, R S Sharma, and S P Tripathi, *Curr Sci*, 1983, **52**, 410
26 E De Clercq, *Antiviral Res*, 1987, **7**, 1
27 G T Tan, A Wickramasinghe, S Verma, R Singh, S H Hughes, J M Pezzuto, M Baba, and P Mohan, *J Med Chem*, 1992, **35**, 4846
28 R I Brinkworth and D P Fairlie, *Biochem Biophys Res Commun*, 1992, **188**, 624
29 H K Wang, J X Xie, J J Chang, K M Hwang, S Y Liu, M B Lawrence, J B Jiang, and K H Lee, *J Med Chem*, 1992, **35**, 2717