

Plant Metabolites Active against *Trypanosoma cruzi*

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Abstract: Parasitic diseases are widespread in less developed countries, and are a major cause of suffering and inability of the affected populations to improve their own living conditions. Among these diseases, American trypanosomiasis (Chagas' disease), due to the kinetoplastid protozoon *Trypanosoma cruzi*, is particularly relevant to Latin America. The natural products literature mentions a wide variety of isolated substances showing activity against this parasite. Although some of these compounds appear to be promising leads, their potential is presently limited by the need of high concentrations, unfavorable pharmacokinetics, and/or by their low solubility in blood. Their mechanisms of action are unknown in most cases, although some trends appear to be emerging. This review presents and discusses the data available until mid-1995.

Key words: Antiprotozoal, natural products, *Trypanosoma cruzi*, Chagas' disease.

Introduction

Chagas' disease (American trypanosomiasis) is a complex clinical entity caused by the flagellate protozoon *Trypanosoma cruzi* (Kinetoplastida, Trypanosomatina). This microorganism, which is able to infect a broad range of vertebrates, is a mostly intracellular parasite transmitted in Nature by blood-sucking triatomine bugs. It has a multistage life cycle with different developmental stages in different hosts. In mammals, *T. cruzi* multiplies intracellularly as (nonflagellate) amastigotes and is subsequently released into the bloodstream as trypomastigotes. These are non-dividing forms that can infect new host cells or be ingested by the insect vectors. In the bug's midgut lumen, trypomastigotes differentiate to epimastigotes, which are the forms that multiply in invertebrates. In the insect rectum, epimastigotes differentiate again to the non-dividing metacyclic trypomastigotes, which are discharged in the excreta while the bug feeds. These infective forms can reach the bloodstream of the vertebrate host through discontinuities of the skin and mucous membranes, and then penetrate cells, thus completing their biological cycle (1). Between 16 and 18 million people are estimated to be infected with *T. cruzi* from Mexico to central Chile and southern Argentina (2), and some concern has arisen lately regarding the situation in the USA (3)

where between 50,000 and 100,000 infected persons, mainly immigrants from Central America, may now be living.

In its acute phase, Chagas' disease is seldom fatal and resolves spontaneously in a few weeks. Nevertheless, the patients remain parasitemic and the infection may be transmitted by insects or by blood transfusion. Many years after acquiring the infection, up to 30% of the patients develop symptomatic disease which in most cases affects the heart, leading to death from dysrhythmias or congestive heart failure, or the gut, leading to sometimes fatal megaesophagus or megacolon. Very little is known about the mechanisms underlying this pathology, although autoimmune processes involving tissue destruction by the patient's lymphocytes have been implicated. These parasites are known to produce a unique trans-sialidase (neuraminidase) which is able to transfer sialic acid residues between different substrates (4), as well as hydrolyzing the neuraminic acids of the glycoproteins on the surface of immune system cells (5), thus possibly initiating the described damage and facilitating invasion of the mammalian cells (6).

The transmission of Chagas' disease by transfusion of infected blood has become a major health problem in South and Central America (7). The only trypanocidal substance currently used to prevent infection by this route is crystal violet, but its use is limited due to its toxic effects (8, 9), and the alarming color communicated to the skin and urine of transfusion recipients. It has been suggested that some antioxidants frequently used in the food industry such as, for example, BHT (2,6-di-*tert*-butyl-4-hydroxytoluene) and BHA (2-mono- and 2,6-di-*tert*-butylated 4-hydroxyanisoles), may be suitable substitutes for crystal violet in the sterilization of blood for transfusion (10), and it also seems likely that some natural products with recognized low toxicity and potent antioxidative qualities may be more appropriate than synthetic antioxidants if their trypanocidal activity against the infective but non-dividing blood form of *T. cruzi* proves to be adequate (11).

Most of the synthetic compounds presently used to treat parasitic diseases produce toxic side effects. Thus, the nitroheterocycles nifurtimox and benznidazole, the only drugs used in the early stages of American trypanosomiasis (which usually go undetected), although practically useless in the advanced disease, owe their action to the reduction of a nitro group to the corresponding anion radical. This also appears to be the cause of the toxic reactions observed in about 50% of the patients (12, 13). For this reason, a continued search for trypanocidal sub-

stances, coupled with studies on their mechanisms of action to facilitate the rational development of lead compounds, is an excellent justification for ongoing screening of natural products active against *T. cruzi* and related organisms.

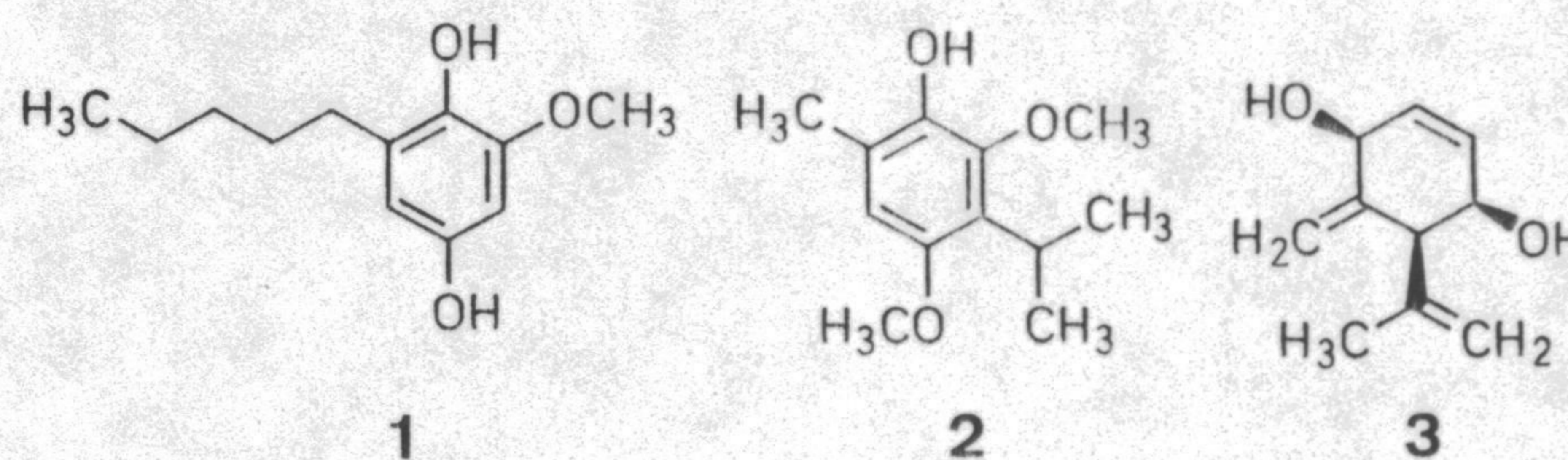
Trypanosomatid parasites are known to be very sensitive to alterations in their redox balance (14–16). Their first line of defense against peroxidative damage, as in their mammalian hosts, consists of reduced glutathione (GSH). Unlike mammals, however, they lack glutathione reductase (GR) and keep their GSH reduced by non-enzymatic reaction with reduced trypanothione (T[SH]₂, 18,-bis-glutathionylspermidine) and 1-glutathionylspermidine. The latter thiol compounds are kept in their reduced forms by trypanothione reductase (TR), an enzyme specific to trypanosomatids. GR and TR are unable to catalyze the reduction of each other's natural substrate(s) (17). It has recently been shown that the readily reducible crystal violet inhibits *T. cruzi* TR by competing for the trypanothione site with $K_i = 5.3 \mu\text{M}$ (18). Considering the likelihood that inhibition of the parasite's TR may be implicated in the effectiveness of nifurtimox and benznidazole, it is worth pointing out that nifurtimox, at least, is a better inhibitor of the host's GR than of the parasite's enzyme (19), thus possibly explaining in part its often intolerable toxicity. As will be seen below, many trypanocidal natural products have structures suggestive of redox activity, and in some cases interference with the function of TR may be the key to their antiparasitic action.

Seeking new chemopreventive and chemotherapeutic agents relevant to Chagas' disease, natural products with a very broad range of structural types have been tested against *T. cruzi* cultures or in infected mammals (20). Many substances with the desired activity have been identified, but their potential usefulness is limited in many cases by their low potency, bioavailability, and/or solubility in blood, pointing to the need of rational, preferably mechanism-based structural manipulations which, in general, are lacking. In the following review of the literature, effective concentrations have been quoted as a guide. Nevertheless it must be noted that, due to the wide variety of experimental conditions used to test these compounds, any comparisons must be made with extreme caution.

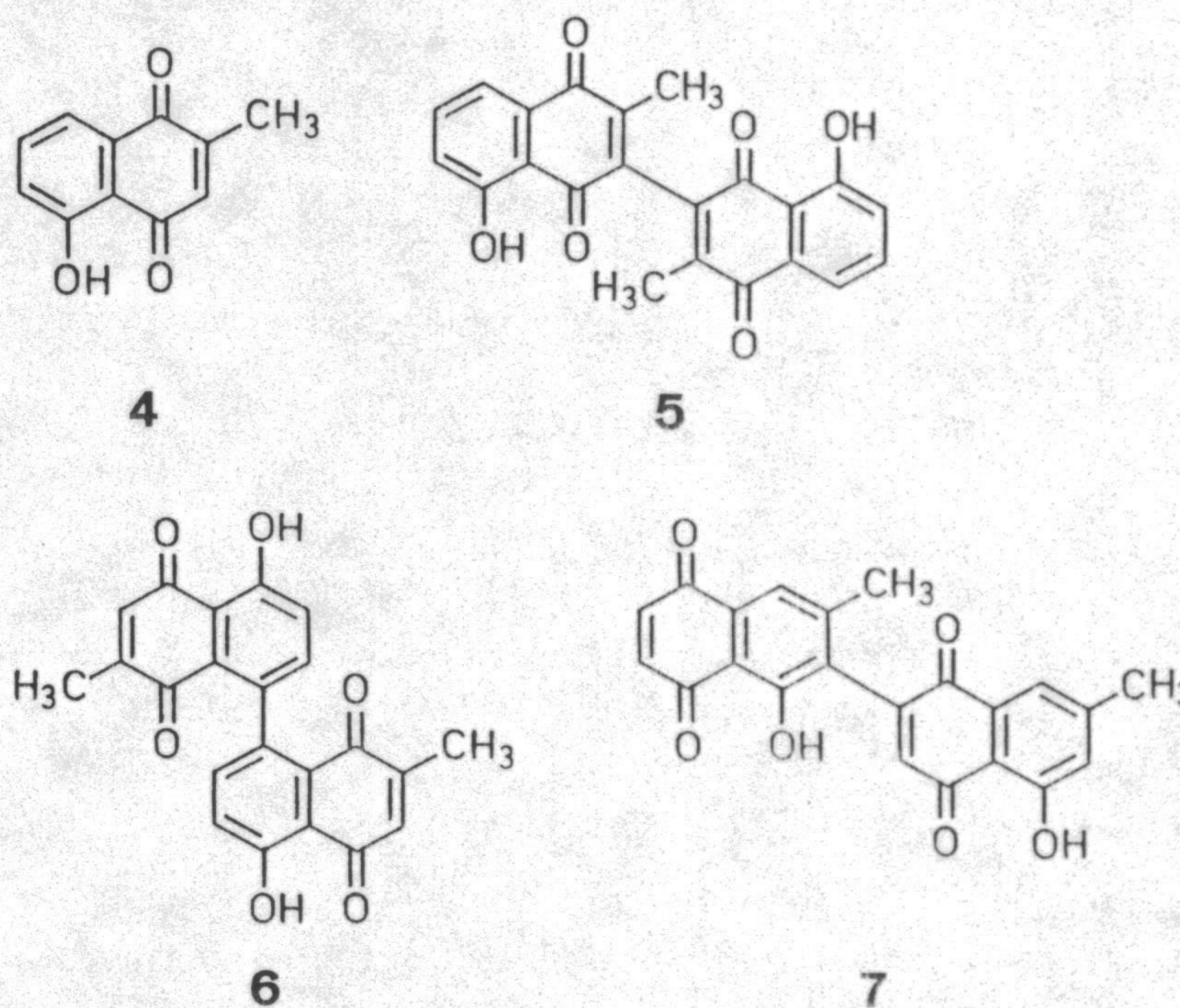
Active Plant Metabolites

Three particularly simple examples of trypanocidal natural products are the hydroquinone derivative miconidin (1), and the monoterpenes espintanol (2) and piquerol A (3). Quantitative data are not available for miconidin (21). Espintanol, isolated by bioassay-guided fractionation of *Oxandra espintana* (22), exhibited IC₉₀ in the 25–100 $\mu\text{g}/\text{ml}$ range against twenty different strains of *T. cruzi* epimastigotes in 48 hour culture experiments. Piquerol A was active at about 100 $\mu\text{g}/\text{ml}$ (23). In view of the sensitivity of trypanosomatids to oxidative stress (14–16), and considering the existence of a number of more complex quinonoid substances with trypanocidal actions, it is attractive to speculate that spontaneous or metabolic oxidation of miconidine, espintanol and piquerol A to the corresponding semiquinone radicals or benzoquinones may be the first step required to exhibit antiparasitic activity, after which reaction of the quinones with appropriate redox enzymes (not only TR, but also enzymes common to parasite and host including, perhaps most significantly, components of the respiratory chain) may lead to enzyme inhibition or to the production of semiquinones capable of reducing dioxygen

molecules to superoxide radical anions (redox cycling) with the subsequent overwhelming of the parasite's redox defenses.

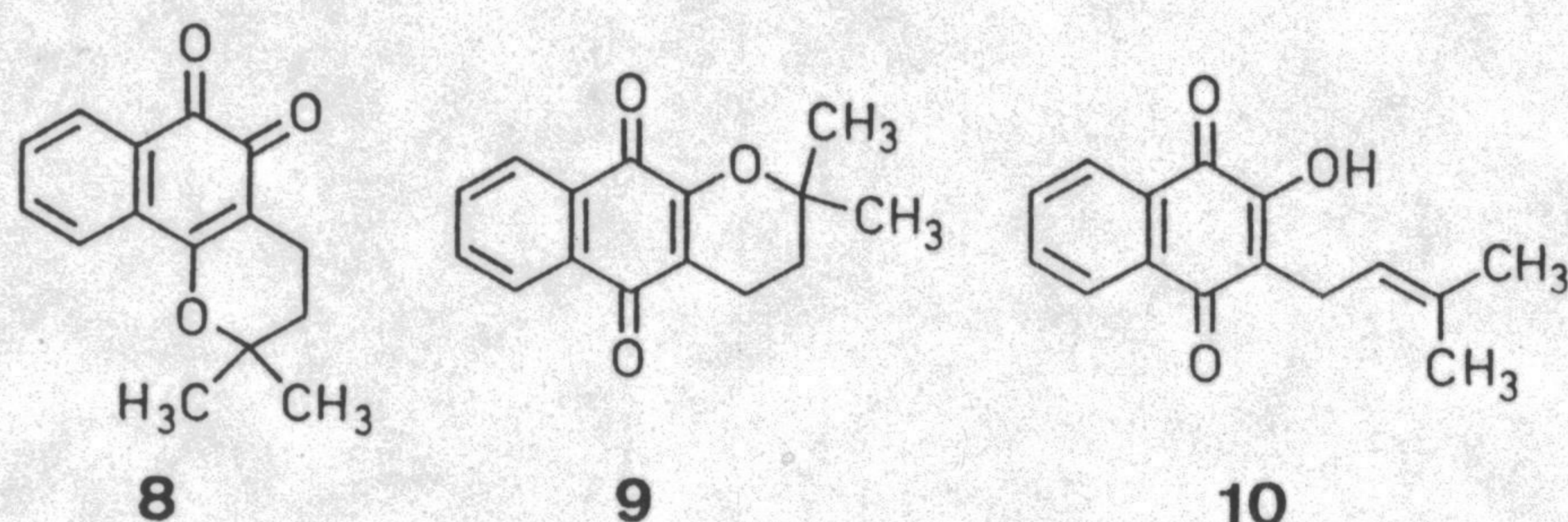


The simple naphthoquinone plumbagin (4) and its dimers, 3,3'-bisplumbagin (5) and 8,8'-bisplumbagin (6), were isolated by bioassay-guided fractionation of *Pera benensis*, used in the treatment of cutaneous leishmaniasis in Amazonian Bolivia (24). Plumbagin, which had previously been shown to be active against *Leishmania* (25), exhibited high potency (IC₉₀ = 1–5 $\mu\text{g}/\text{ml}$) against six strains of *T. cruzi* epimastigotes, while the dimers were less active, with IC₉₀ in the 25–100 $\mu\text{g}/\text{ml}$ range. It is noteworthy, however, that in this assay nifurtimox and benznidazole also showed IC₉₀ values of 25–100 $\mu\text{g}/\text{ml}$. The related diospyrin (7) is also active, and appears to act by inhibiting respiration of the parasite (26). As it stimulates oxygen consumption by *T. cruzi*, it seems likely that this compound (and possibly plumbagin and its dimers) also owes at least part of its effect to the production of reactive oxygen species.

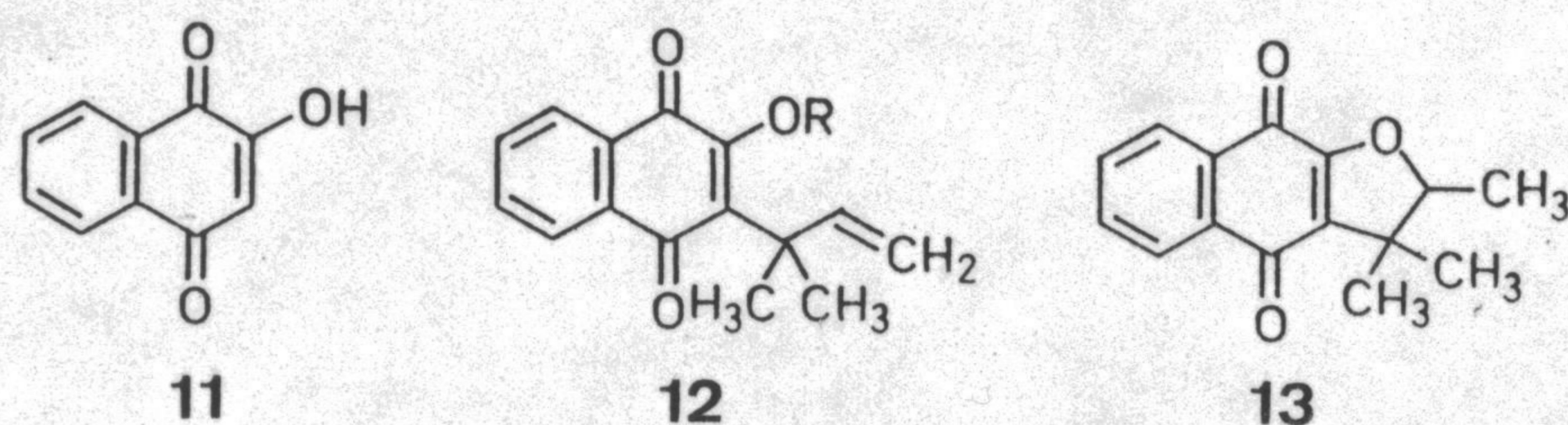


Quinone redox cycling in *T. cruzi* has been shown to generate superoxide anion which, after dismutation to hydrogen peroxide catalyzed by superoxide dismutase, originates the highly cytotoxic hydroxyl radical. β -Lapachone (8), which causes progressive inhibition of epimastigote motility at 5 $\mu\text{g}/\text{ml}$ and has a minimum growth inhibitory concentration of 0.8 $\mu\text{g}/\text{ml}$, was one of the first natural drug molecules to be shown to be reduced to a (1,2-semiquinone) free radical in intact parasite cells in which it leads to enhanced concentrations of highly reactive reduced oxygen species (27, 28). Oxygen free radicals are probably responsible for the irreversible DNA damage observed with 1.6 μM β -lapachone, although this compound also inhibits DNA, RNA and, to a lesser extent, protein synthesis at the same concentration. It is therapeutically

useless, however, because of its rapid inactivation in blood. Somewhat surprisingly, its isomer α -lapachone (**9**) has a minimum growth inhibitory concentration greater than 10 $\mu\text{g/ml}$. The closely related lapachol (**10**) is also weakly active against epimastigotes, with a minimum growth inhibitory concentration of about 10 $\mu\text{g/ml}$. Synthetic 3-allyl- β -lapachone, however, is effective against trypomastigotes (presumably via redox cycling) at 1.0 $\mu\text{g/ml}$, is not inactivated in blood, and has been considered as a possible chemoprophylactic for use in blood banks (28). Nevertheless, it is detoxified by mammals and therefore cannot be used for treating patients infected with *T. cruzi* (29). As trypanosomatids are deficient in protective mechanisms against oxygen radicals (30), Stoppani's team looked for other compounds capable of generating such active species and observed similar behavior in *Crithidia fasciculata* and *Leptomonas seymouri* with several other 1,2-naphthoquinones including the natural mansonones A, C, E, and F which, unfortunately, were not tested against *T. cruzi*, and three synthetic β -lapachone analogues (31).

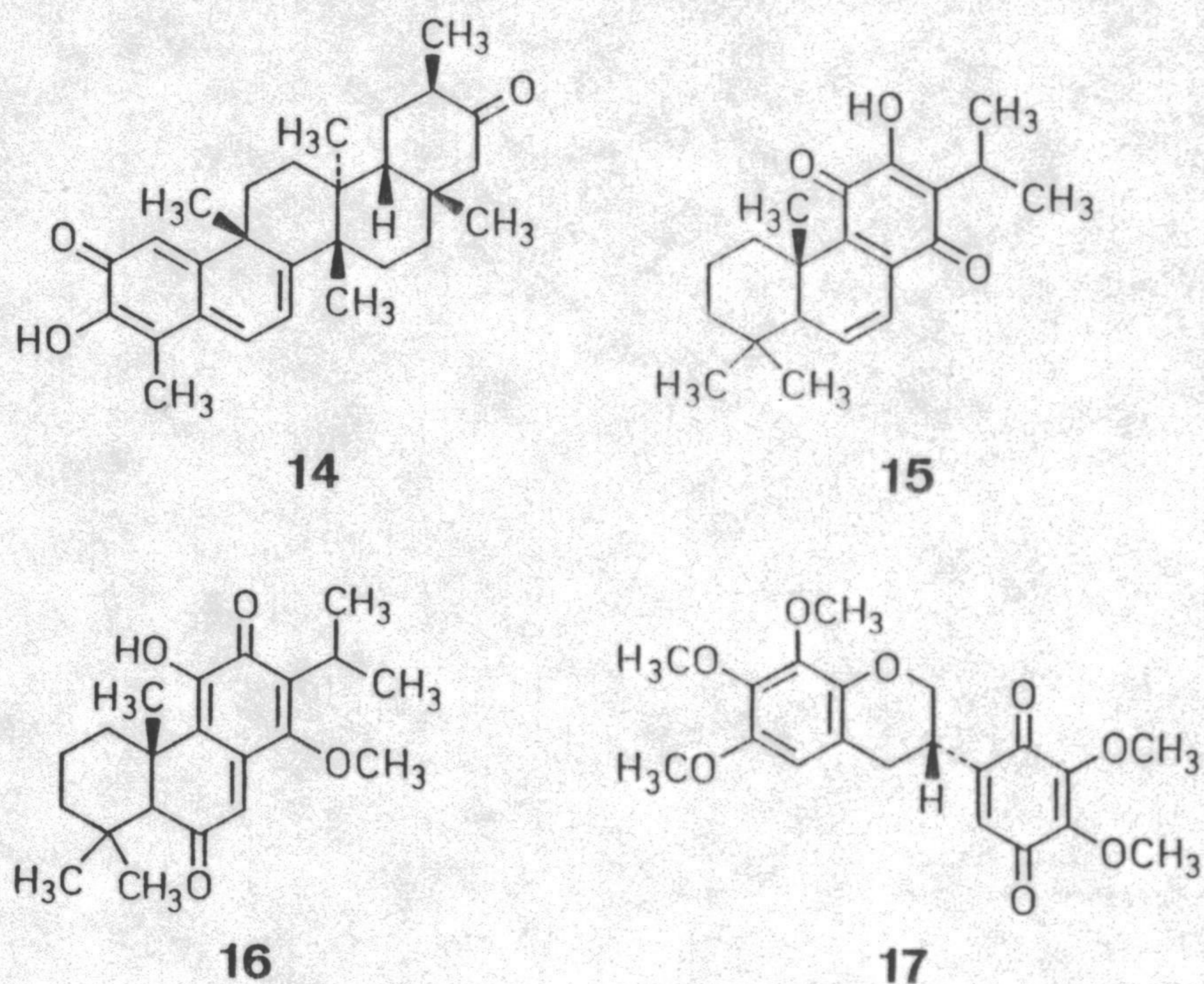


Lawsonone (2-hydroxy-1,4-naphthoquinone, **11**), 3-(1,1-dimethylallyl)-lawsonone (**12**, R = H), the acetate of the latter (**12**, R = CH₃CO), and the cyclized α -lapachone analogue (-)-2,3,3-trimethyl-2,3-dihydronaphtho[2,3-*b*]furan-4,9-quinone (**13**) inhibited the proliferation of *T. cruzi* epimastigotes in culture with IC₅₀ (for three different strains) in the ranges 20–50, 4.2–8.4, 5.3–15.1, and 2.1–5.2 μM , respectively (from 3.5–8.7 down to 0.5–1.3 $\mu\text{g/ml}$). The most potent of these compounds (**13**), comparable in this regard to plumbagin and β -lapachone, which inhibited the respiration of tumor cells by interfering with electron transport at an early stage, did not inhibit respiration of *T. cruzi* but produced a temporary increase in oxygen consumption suggestive of redox cycling (32).

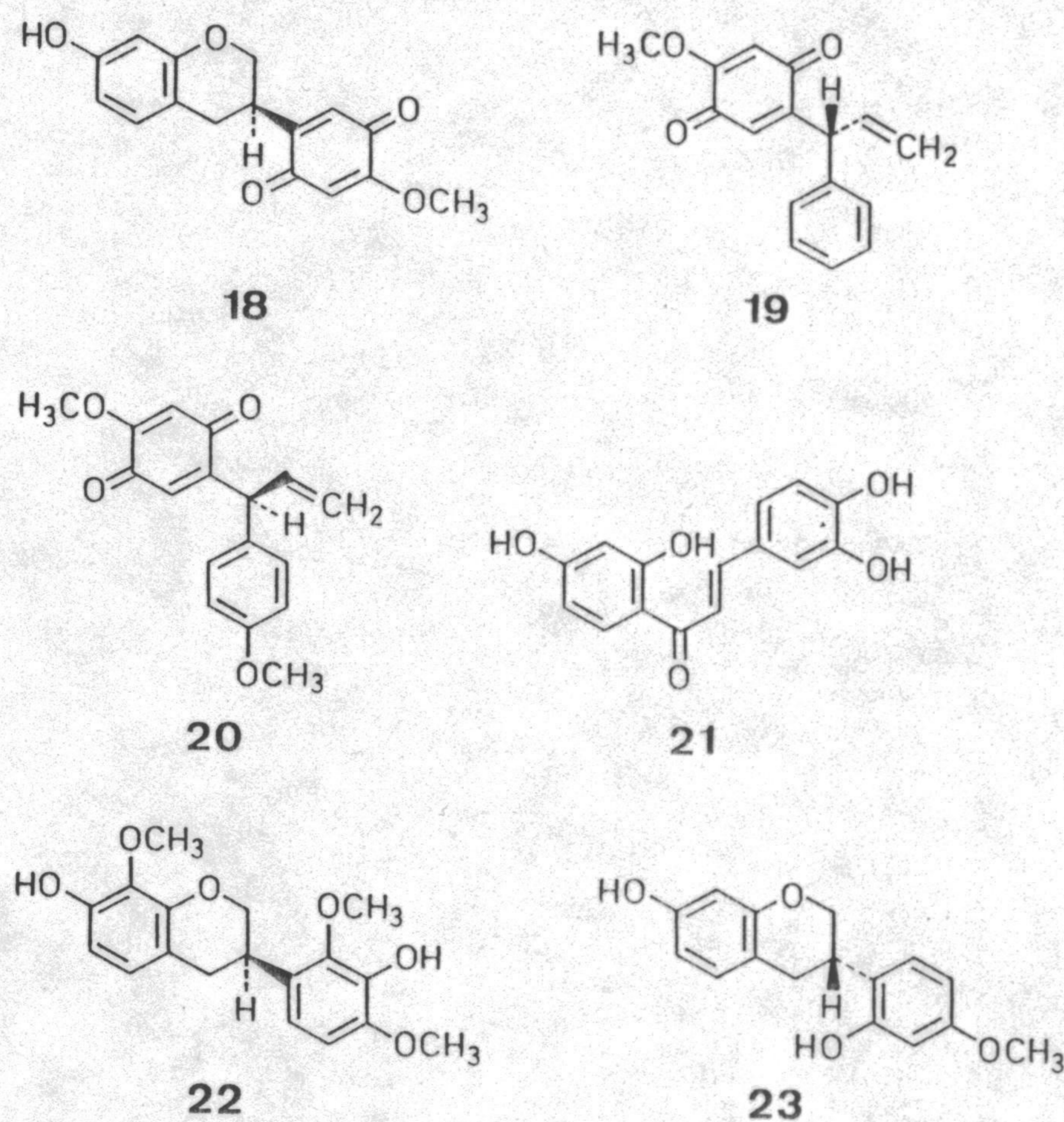


The unusually well studied antineoplastic quinonoid triterpene tingenone (**14**) has been shown to be as effective against *T. cruzi* epimastigotes *in vitro* as nifurtimox, and more so than benzimidazole (I₅₀ = 12 and 40 μM , respectively). 30 μM Tingenone (12 $\mu\text{g/ml}$) completely inhibited the growth of the parasite. Tingenone blocks mitochondrial electron transport, but this does not appear to contribute to the overall action of the drug on the parasite. In fact, horminone (**15**), which is ten times as active as tingenone as an inhibitor of electron transport, is no more potent as an inhibitor of the proliferation of *T. cruzi* (33). Tingenone does not produce reactive oxygen species as is the case for other quinonoid compounds. On the other hand, it in-

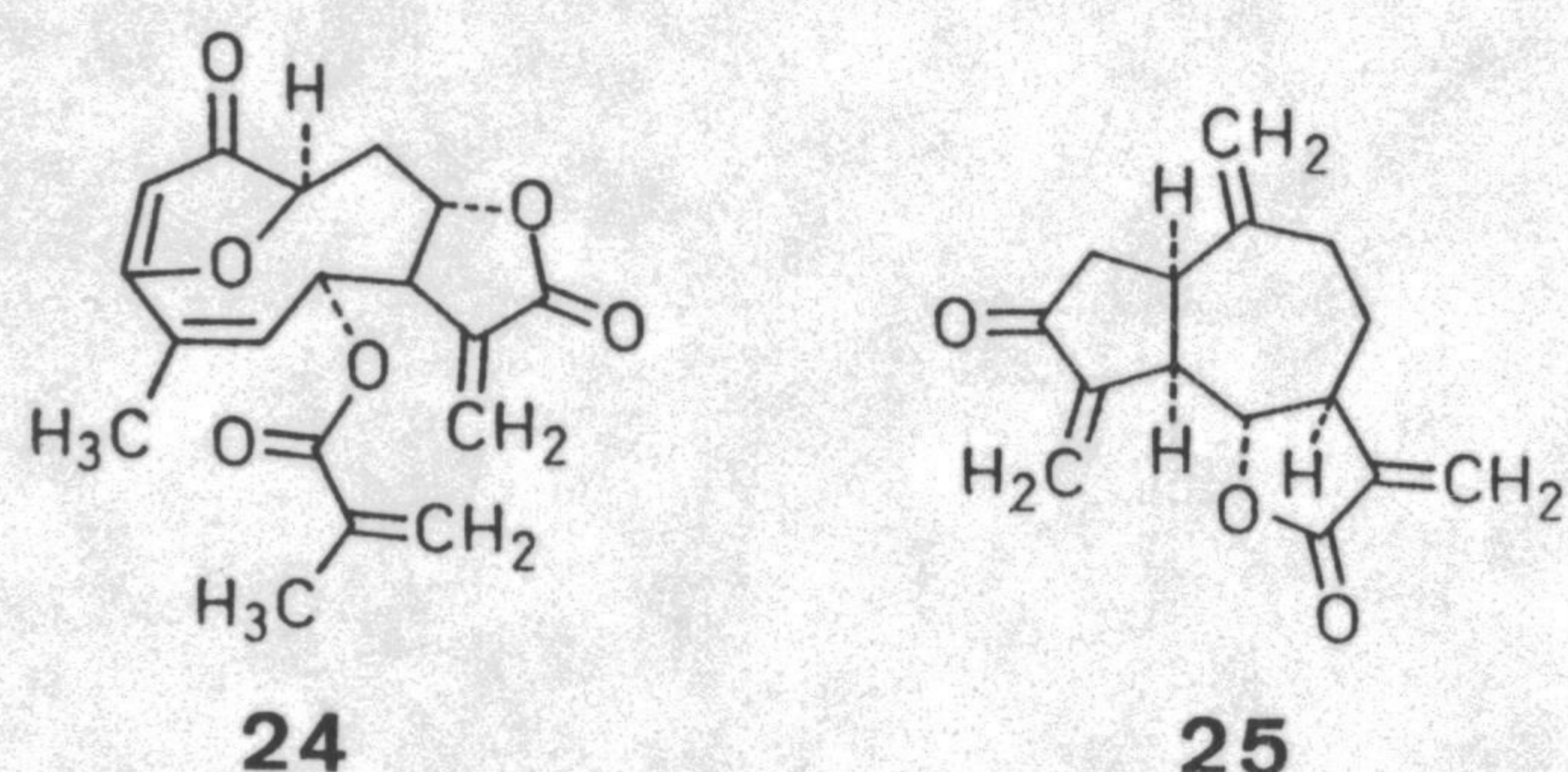
hibited total uptake and incorporation of [³H]-thymidine, [³H]-uridine, and L-[³H]-leucine into protozoal macromolecules (34, 35), leading to the death of the parasite. Similarly, the diterpenoid 14-methoxytaxodione (**16**) and the isoflavonoid abruquinone (**17**) lysed *T. cruzi* at 500 μM (>100 $\mu\text{g/ml}$), a rather low potency in comparison with other substances (33, 34).



It has been shown that the flavonoid quinones (3*R*)-claussequinone (**18**), (*R*)-4-methoxydalbergione (**19**), and (*S*)-4,4'-dimethoxydalbergione (**20**), lyse *T. cruzi* trypomastigotes *in vitro* at concentrations around 100 μM (about 25 $\mu\text{g/ml}$) (20). The related non-quinonic (but possibly quinone precursors) butein (**21**), (3*R*)-duartin (**22**), and (3*S*)-vestitol (**23**) only exhibited effectiveness toward some of the different parasite strains tested, and only at 2 mM concentration (20).



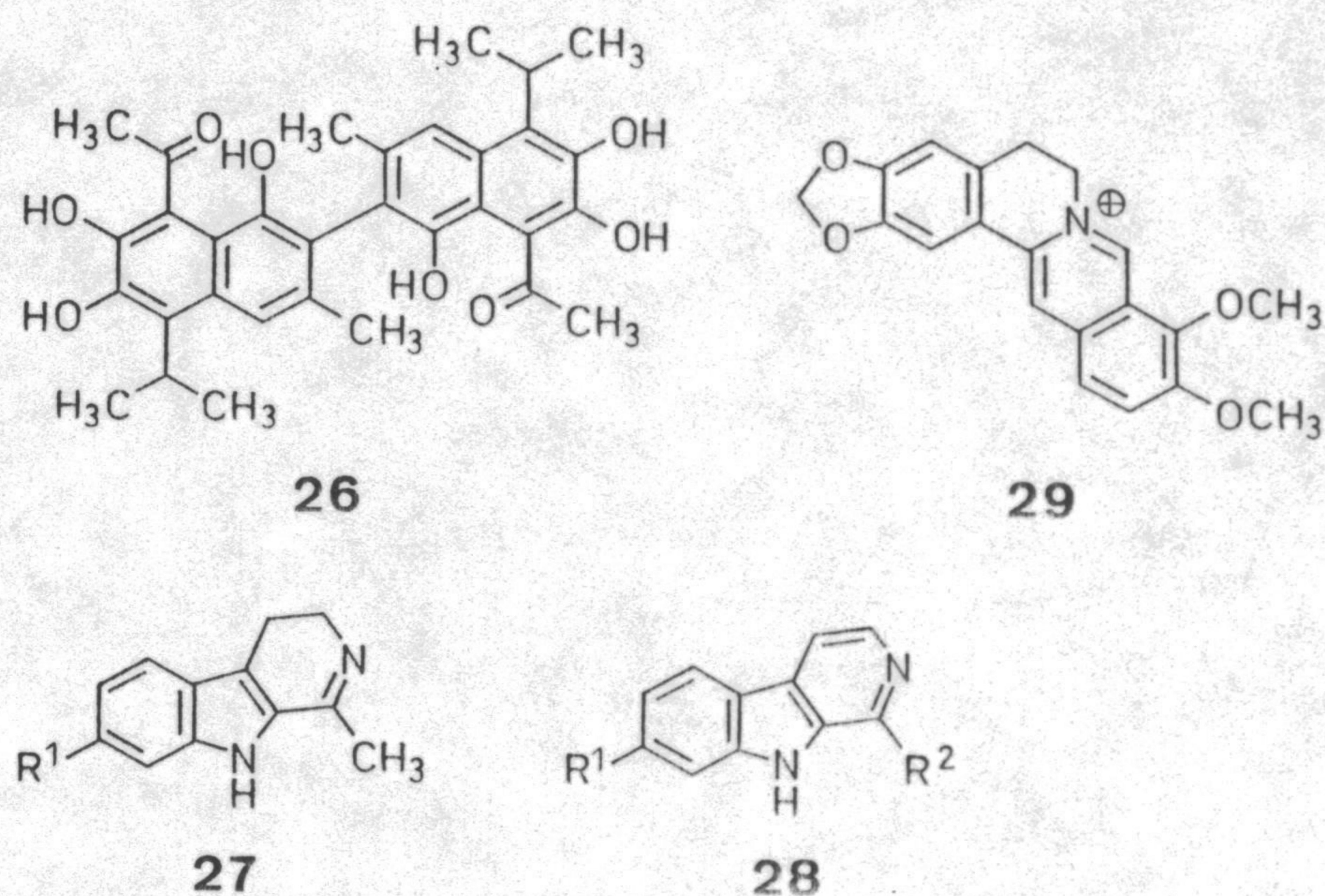
The electrophilic but probably not redox-active sesquiterpene lactone 15-deoxygoyazensolide (**24**) also destroyed the parasite cells at this fairly high concentration (20). In the latter case, the mechanism by which toxicity is expressed is presumably different from those mentioned above and may be related to the widely observed cytotoxicity of α,β -unsaturated lactones. The same is probably true for dehydrozaluzanin C (**25**), which showed IC_{90} in the more attractive 2.5–50 $\mu\text{g}/\text{ml}$ range (10–200 μM) against fifteen different strains of *T. cruzi* epimastigotes in culture (36).



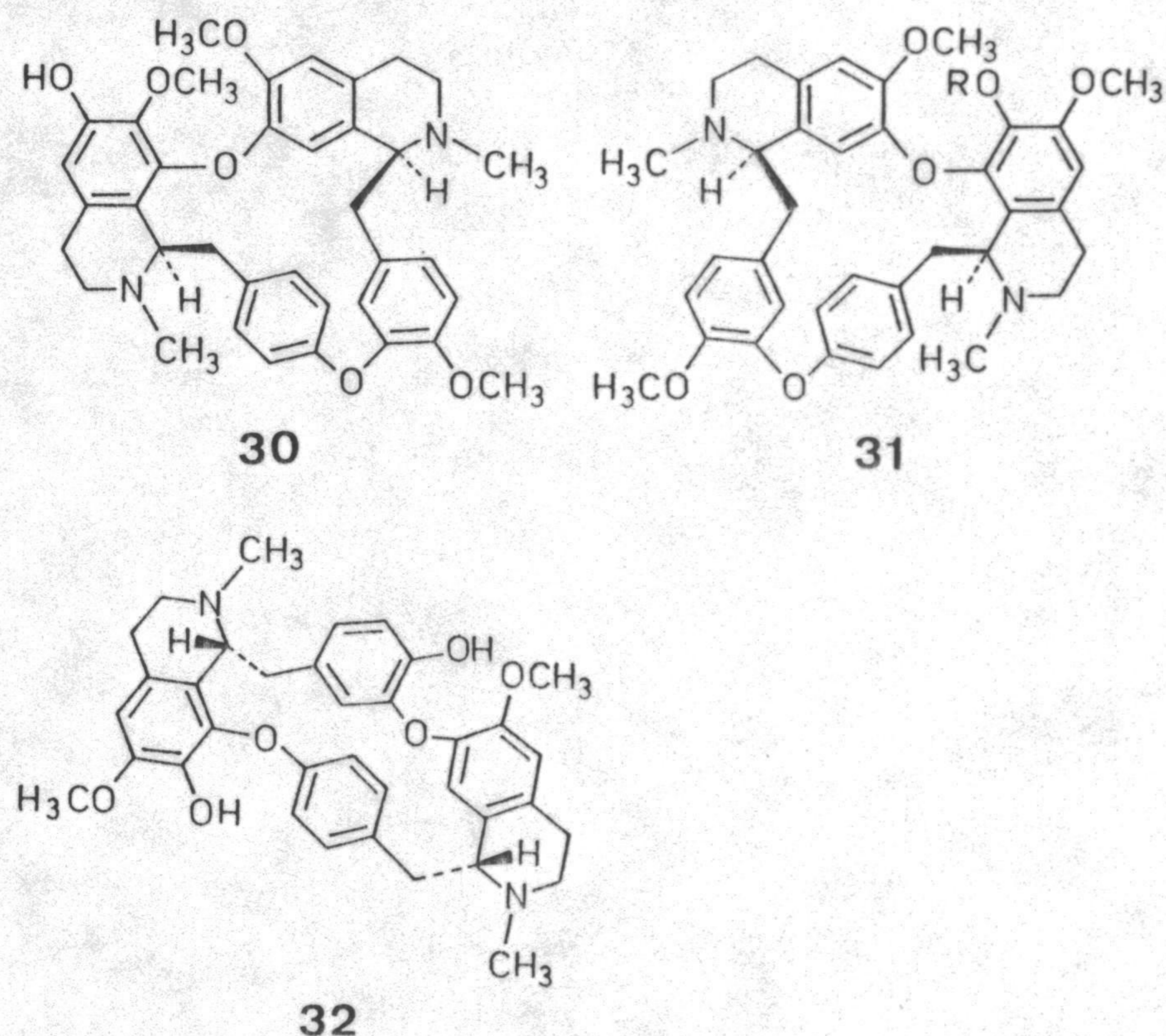
Gossypol (**26**) showed activity at 25–50 μM concentration against the epimastigote form of *T. cruzi*, possibly due to its interaction with DNA (37), or by inhibiting the NADH oxidoreductases α -hydroxyacid dehydrogenase and malate dehydrogenase (38). It is inactivated by blood proteins, however, and is therefore useless therapeutically (39). It may be significant that this compound can be easily oxidized to an orthoquinone which may be the basis of its interference with the parasite's redox balance.

The β -carboline alkaloids harmalol (**27**, $R^1 = \text{OH}$), harmaline (**27**, $R^1 = \text{OCH}_3$), harman (**28**, $R^2 = \text{CH}_3$, $R^1 = \text{H}$), harmol (**28**, $R^2 = \text{CH}_3$, $R^1 = \text{OH}$) and harmine (**28**, $R^2 = \text{CH}_3$, $R^1 = \text{OCH}_3$), inhibit *T. cruzi* epimastigote growth *in vitro* by 50 to 90% at 50 $\mu\text{g}/\text{ml}$ (about 230 μM) or, in the case of harmine, by 30% at concentrations ten times lower (40). On the basis of their structures, even these substances may be presumed to have mechanisms of action related to redox activities. Their possible intercalation with DNA (41), or interference with aromatic amino acid metabolism, however, have been postulated previously as bases for their antiprotozoal actions (42). Also, β -carboline alkaloids are known to be monoamine oxidase (MAO), ($\text{Na}^+ + \text{K}^+$)-ATPase, and Na^+ -dependent glutamate uptake inhibitors (42–44), any of which activities might be related to their antiparasitic action. Some of these substances have also been reported to be hallucinogenic at high doses (45). The latter property, taken together with their fairly low potency against *T. cruzi*, may limit their attractiveness as antiparasitic drugs. Nevertheless, norharman (**28**, $R^1 = R^2 = \text{H}$) has recently been found to be about as potent as harmine in the epimastigote screen (46), and these compounds may therefore be considered as possible leads for the development of therapeutically useful substances. The widespread and abundant alkaloid berberine (**29**), a constituent of traditional remedies for the treatment of cutaneous leishmaniasis which forms a redox pair with 8,13-dihydroberberine (47), also inhibits the growth of *T. cruzi* epimastigotes *in vitro* by 75% at 50 $\mu\text{g}/\text{ml}$ (38).

The head-to-head bisbenzylisoquinolines gyrocarpine (**30**), daphnandrine (**31**, $R = \text{H}$) and obaberine (**31**, $R = \text{CH}_3$), the latter two with absolute configuration opposite to that of the former, completely inhibit epimastigote replication at 50 $\mu\text{g}/\text{ml}$ (about

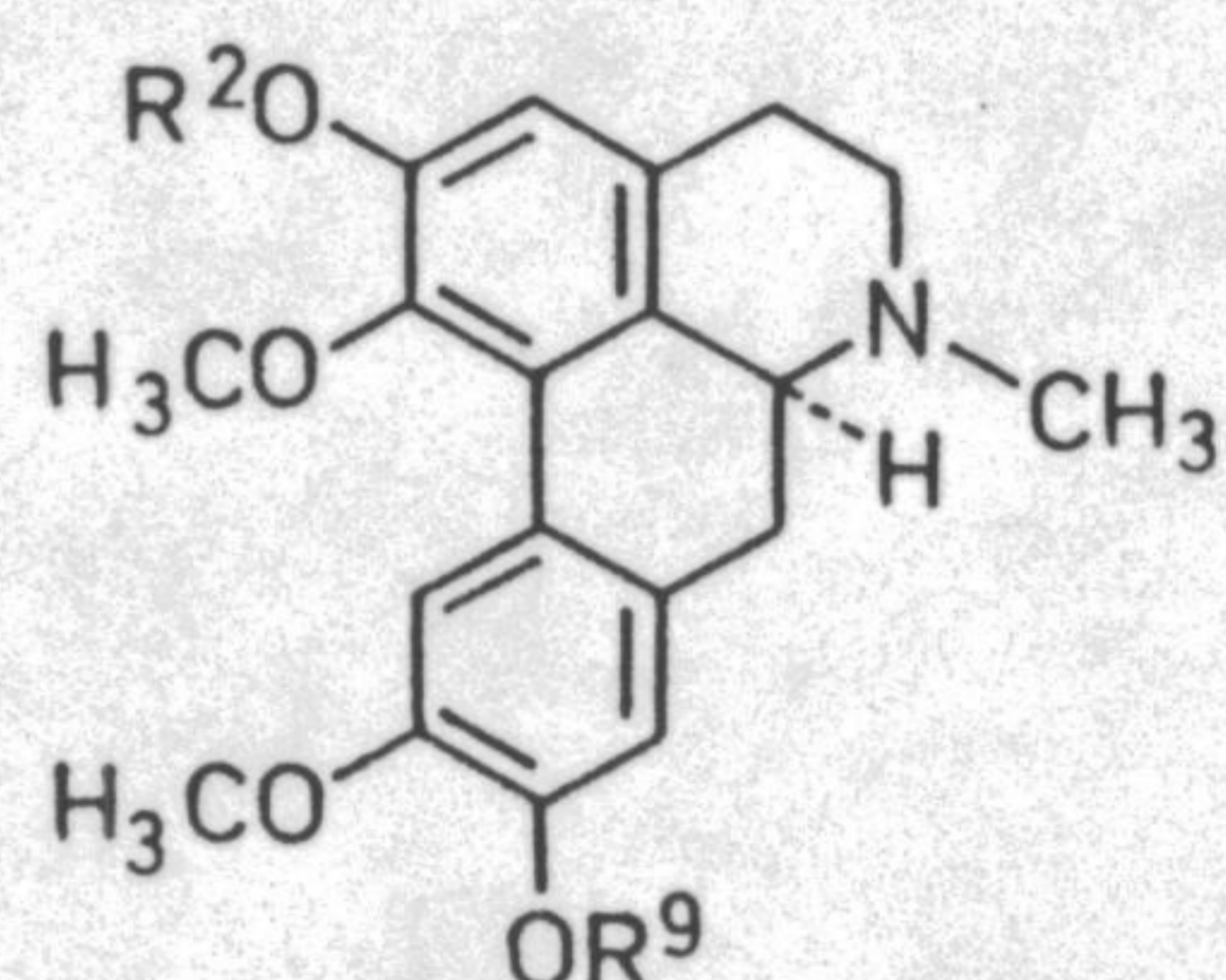


80 μM), and thus appear to be somewhat more potent than nifurtimox and benznidazole in this assay. The head-to-tail dimer chondodendrine (bebeerine, **32**) is only slightly less active, while a wide variety of other bisbenzylisoquinoline alkaloids, some quite closely related to those mentioned, are three to ten times less so in this concentration range (48). These results defy any structure-activity analysis and do not correspond to any identified mechanism of action, although at these high concentrations isoquinoline alkaloids often show monoaminergic and/or Ca^{2+} channel blocking activities.

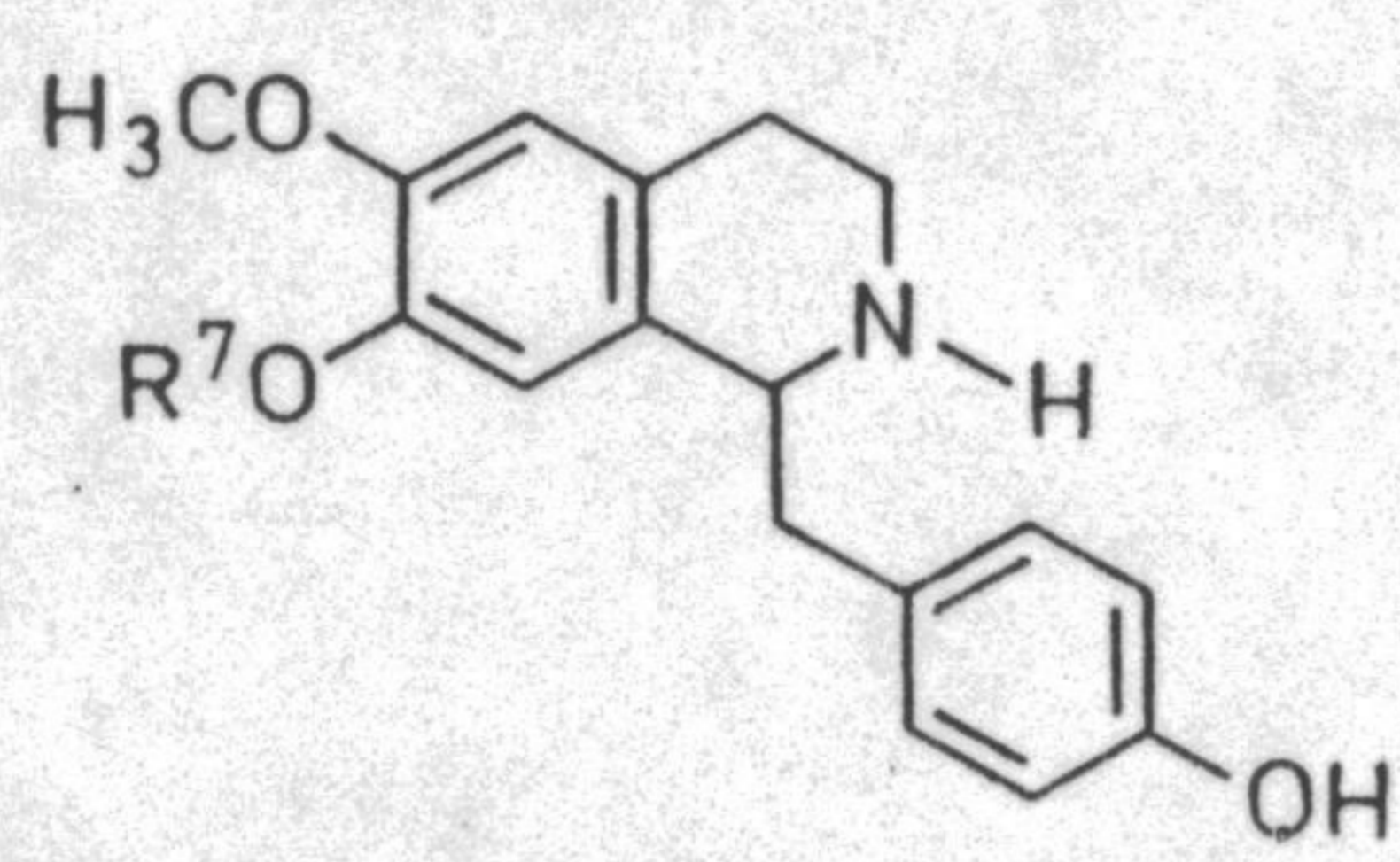


Given the fact that synthetic phenolic antioxidants exhibit activity against *T. cruzi* epimastigotes by inhibiting their respiration (10), it is conceivable that the sterically hindered phenolic groups of the more active bisbenzylisoquinoline alkaloids may be acting as free radical chain breaking antioxidants, and that this property may be related in some way to their anti-trypanosomal actions. This would seem to be the case with the aporphines boldine (**33**, $R^2 = R^9 = \text{H}$), predicentrine (**33**, $R^2 = \text{H}$, $R^9 = \text{CH}_3$), and glaucine (**33**, $R^2 = R^9 = \text{CH}_3$), recently shown to inhibit the growth of *T. cruzi* epimastigotes *in vitro* with IC_{50} of about 30 $\mu\text{g}/\text{ml}$ (100 μM) (11), while the closely related monomeric phenolic benzylisoquinolines (\pm)-coclaurine (**34**,

$R^7 = H$) and (\pm)-norarmepavine (**34**, $R^7 = CH_3$), which are only weak antioxidants (49), are three to five times less active against the parasite, and the nonphenolic (and presumably non-antioxidant) codeine is inactive (11). It seems worth pointing out, however, that the low potency of the monomeric benzyloquinoline alkaloids is no lower than that exhibited by some of the quinonoid compounds mentioned above, suggesting the possibility of developing relatively non-toxic leads derived from the aforementioned chemical class.

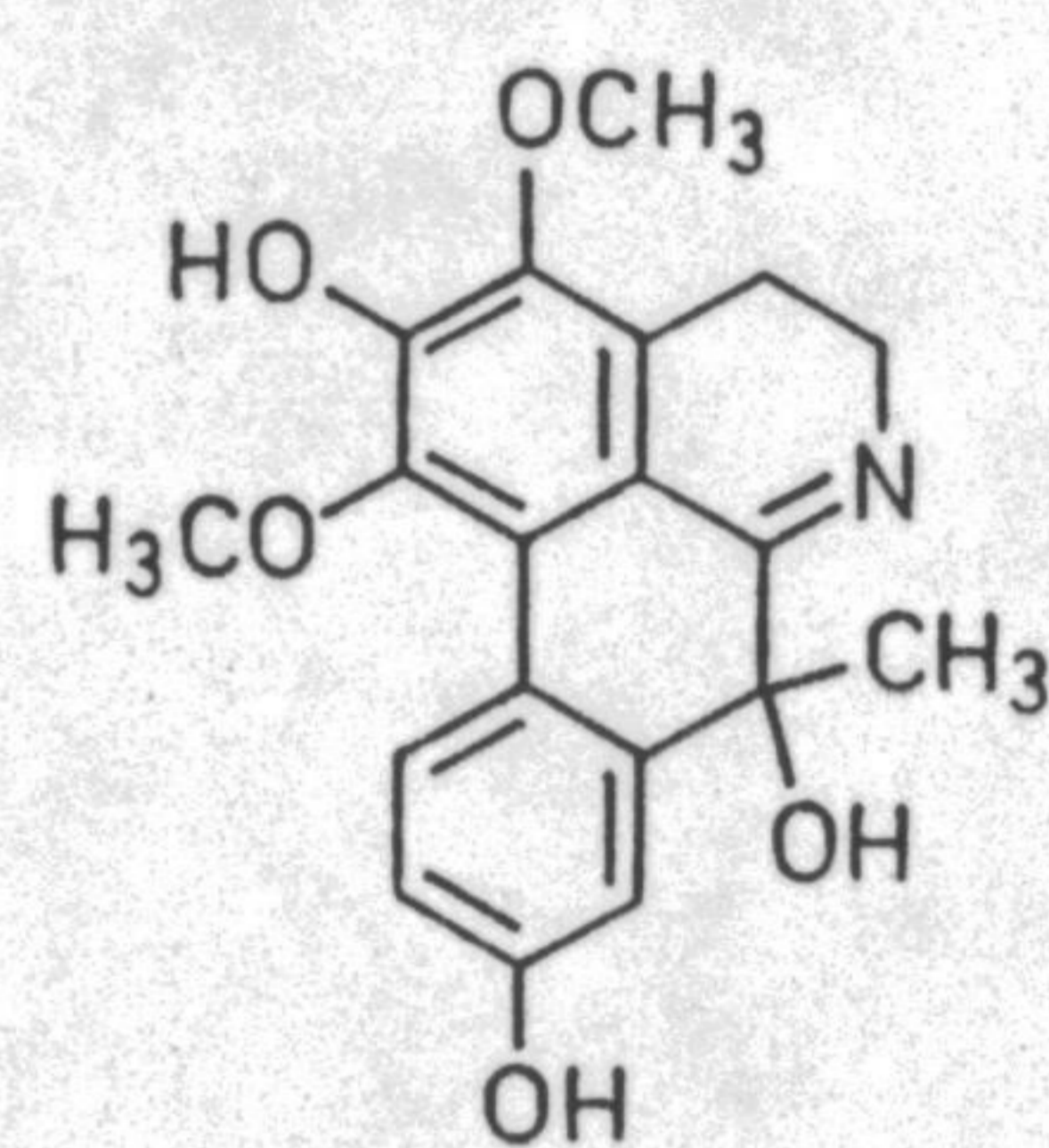


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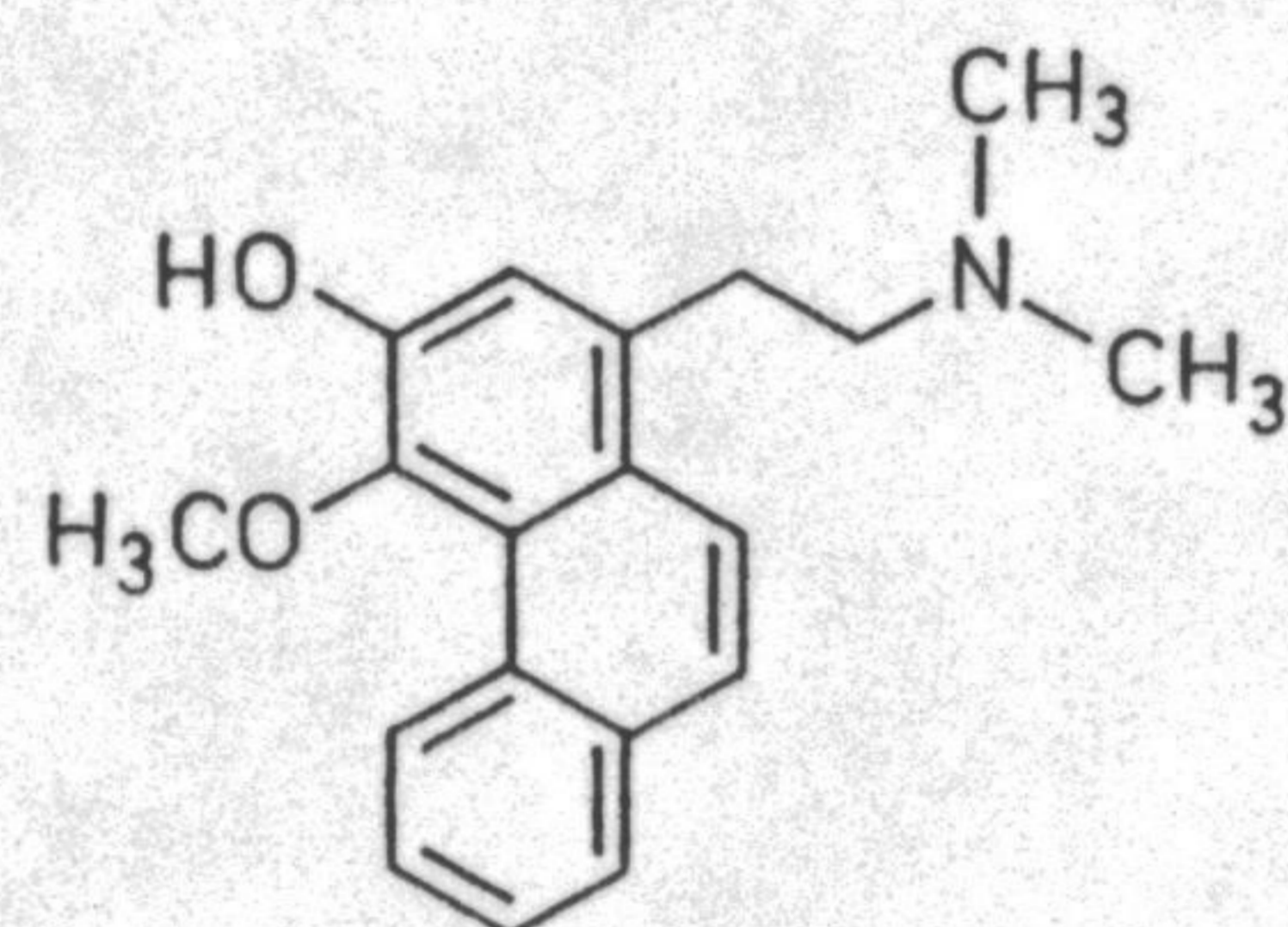


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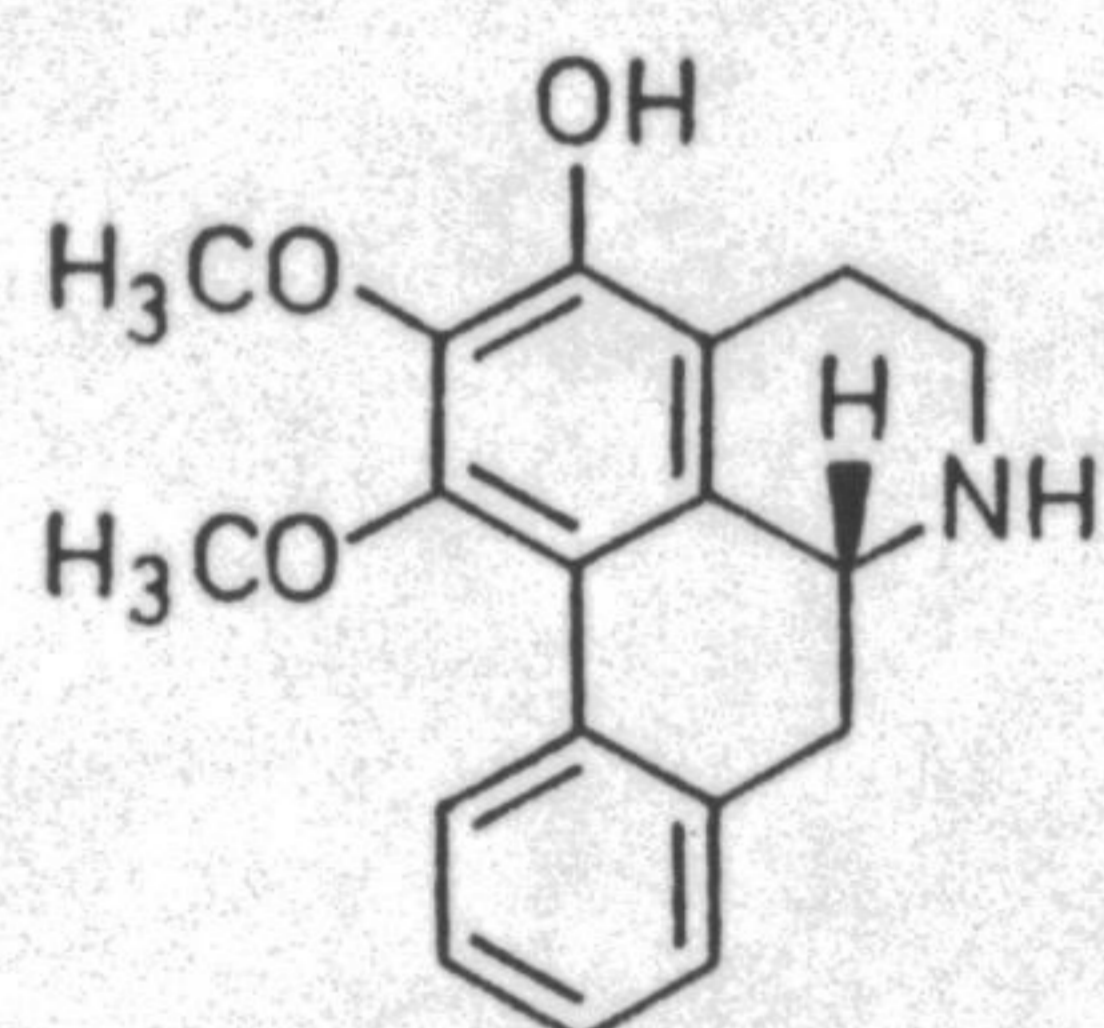
Several additional aporphinoids *sensu lato*, isolated by bioassay (*Leishmania* and *T. cruzi*)-guided fractionation of *Guatteria foliosa* extracts, have been shown to lyse *T. cruzi* trypomastigotes at 250 $\mu\text{g/ml}$ (nearly millimolar concentration). In 24 h experiments, isoguattouregidine (**35**), argentinine (**36**), 3-hydroxynornuciferine (**37**), and 3-methoxyoxoputerine (**38**) caused 92%, 81%, 68%, and 47% lysis, respectively (50).



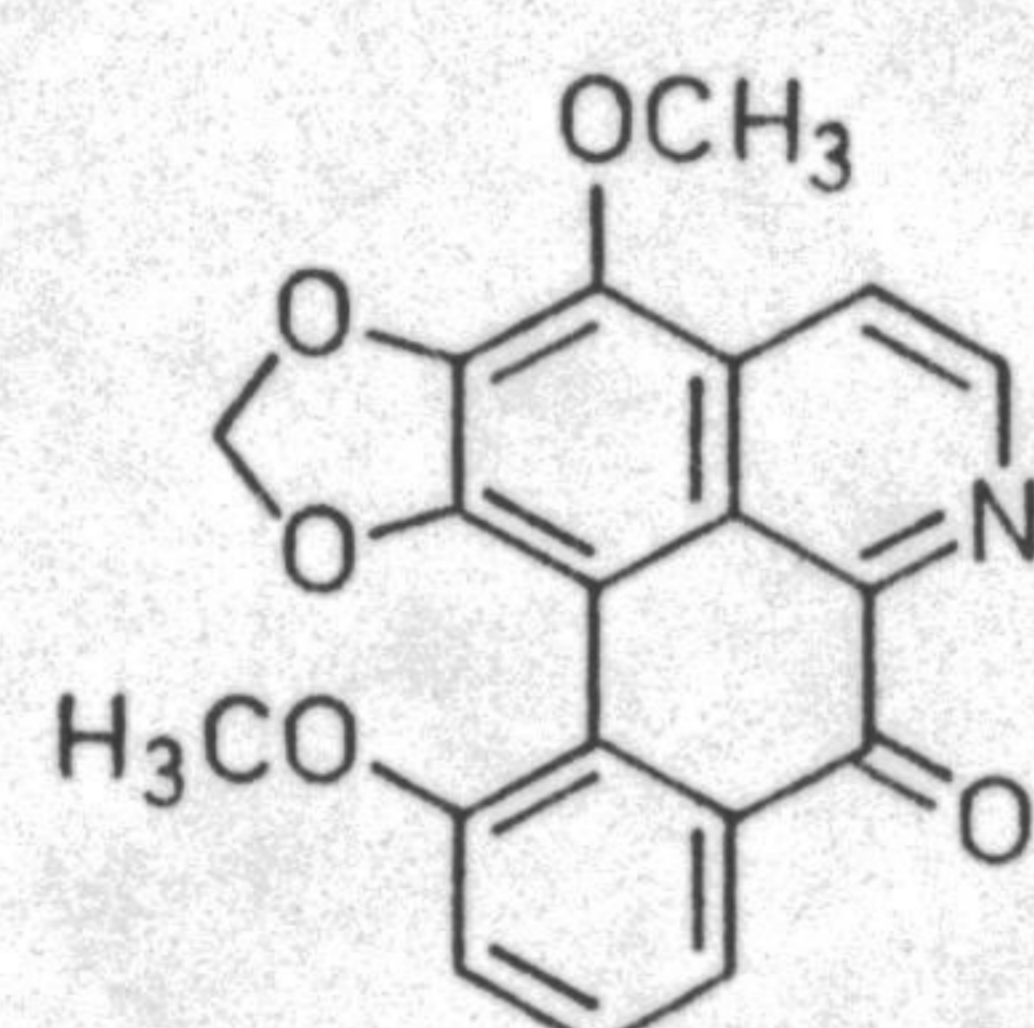
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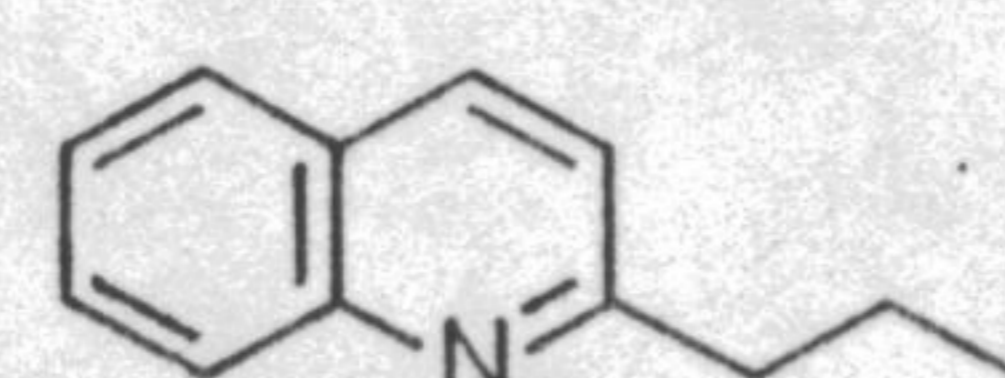
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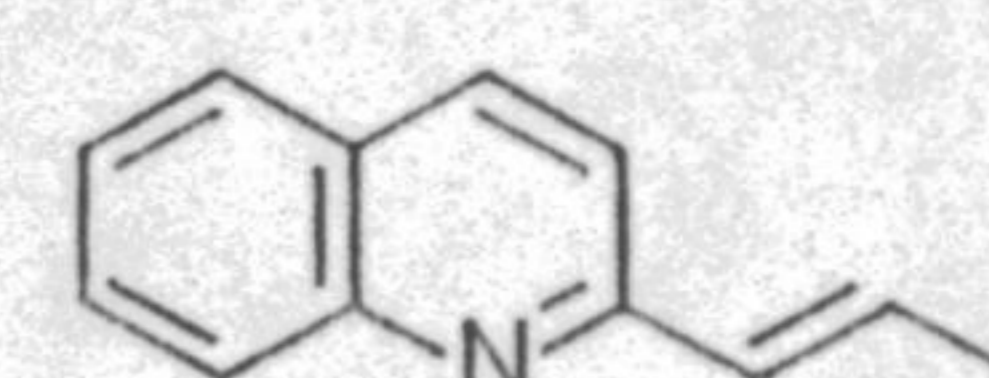
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Interestingly, some simple natural quinoline derivatives such as 2-*n*-propylquinoline (**39**), chimanine B (2-[1(*E*)-propényl]quinoline, **40**), and chimanine D (chimanine B epoxide, **41**), isolated by bioassay (*Leishmania*)-guided fractionation of *Galipea longiflora* used in Northern Bolivia as a remedy for cutaneous leishmaniasis, are about as active *in vitro* as nifurtimox or benznidazole against *T. cruzi* ($IC_{50} = 25-50 \mu\text{g/ml}$ or $150-300 \mu\text{M}$), while other 2-substituted quinolines and the well-known skimmianine (**42**) only show this activity at concentrations two to four times higher (51). No mechanism has been suggested to explain these effects.

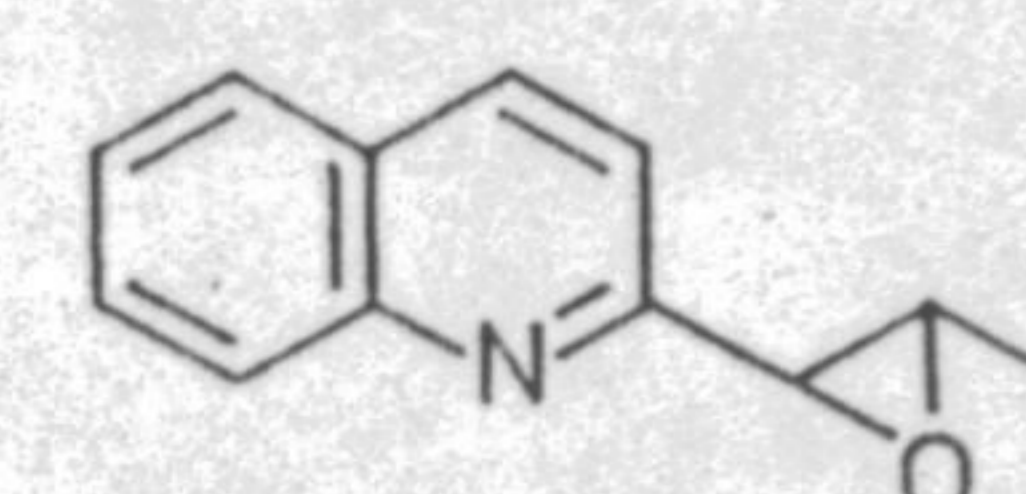
Other alkaloids which have been screened include the traditional antimalarial quinine (**43**) which is rather potent, completely inhibiting *T. cruzi* epimastigote replication *in vitro* at 5 $\mu\text{g/ml}$ ($14 \mu\text{M}$). Interaction with DNA would seem to be a



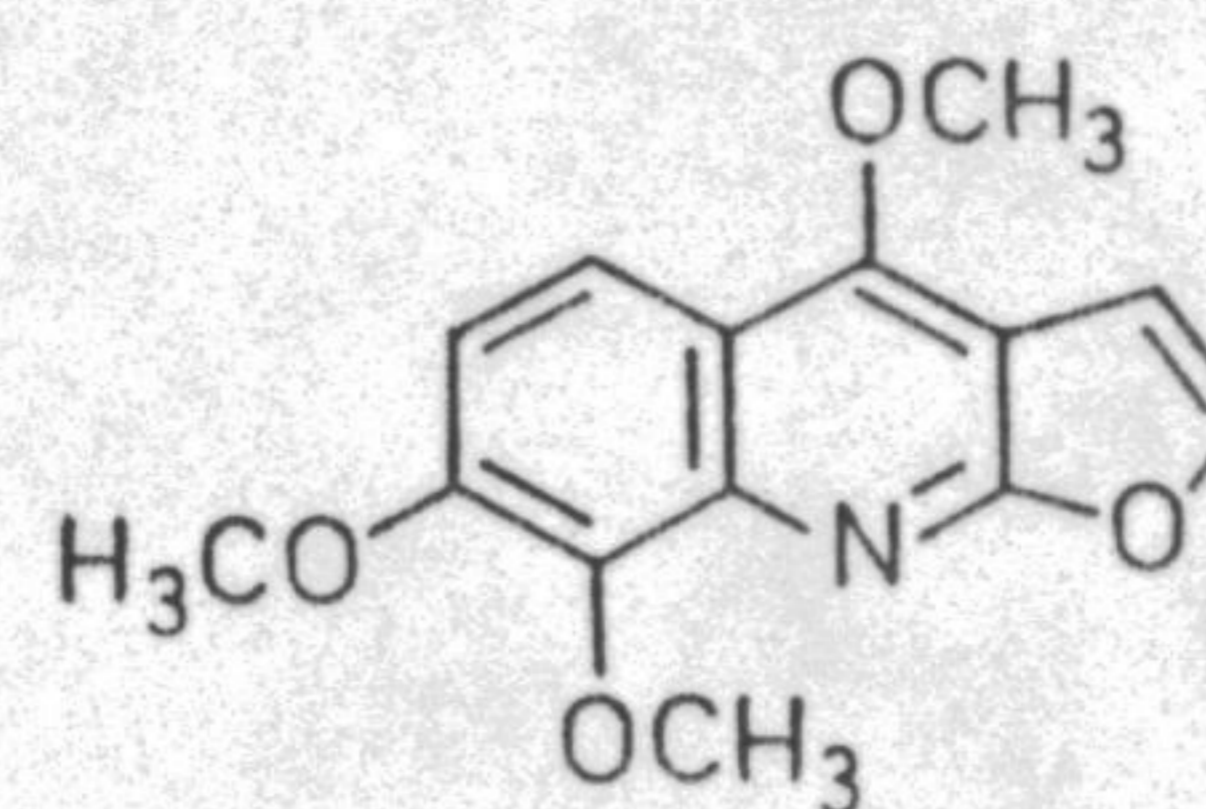
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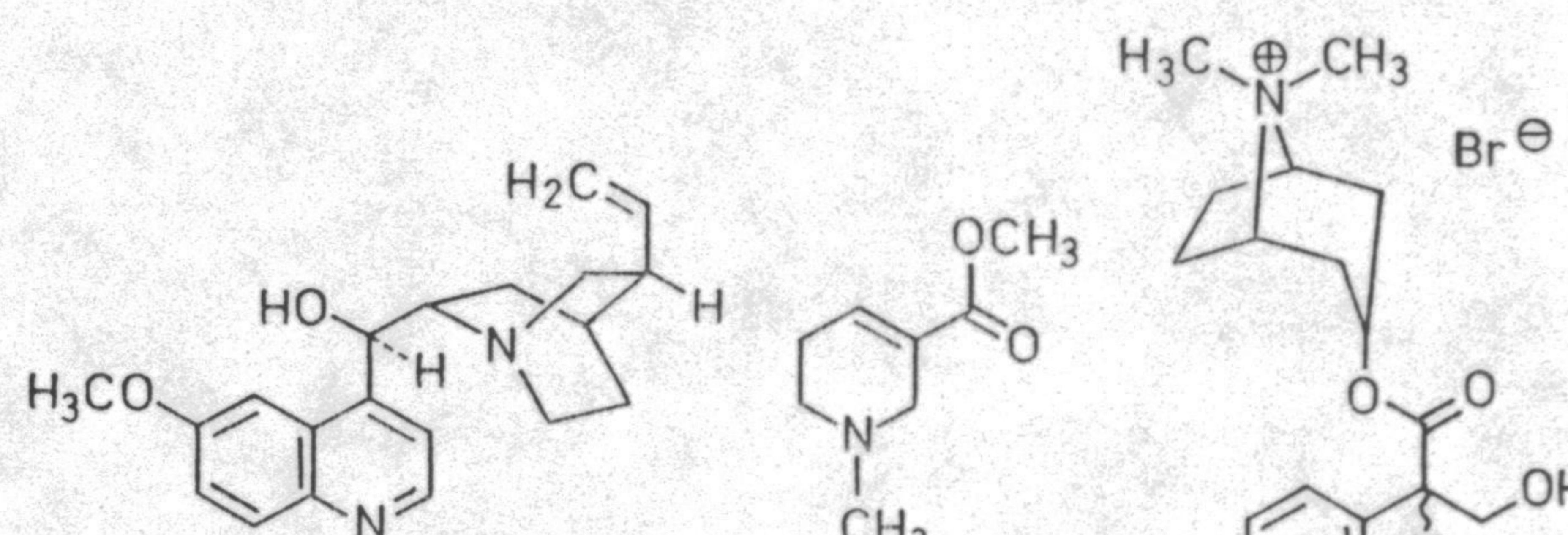


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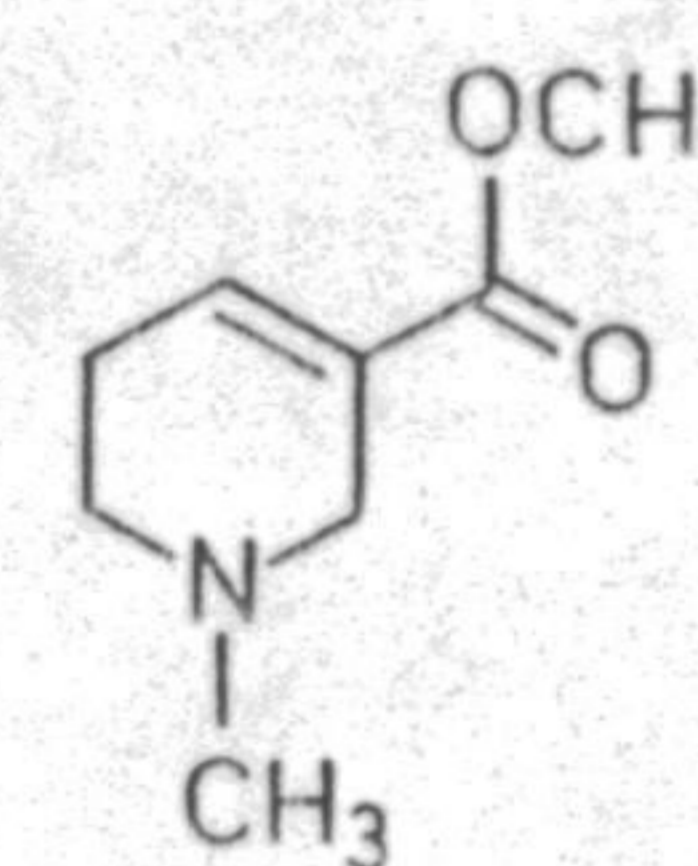


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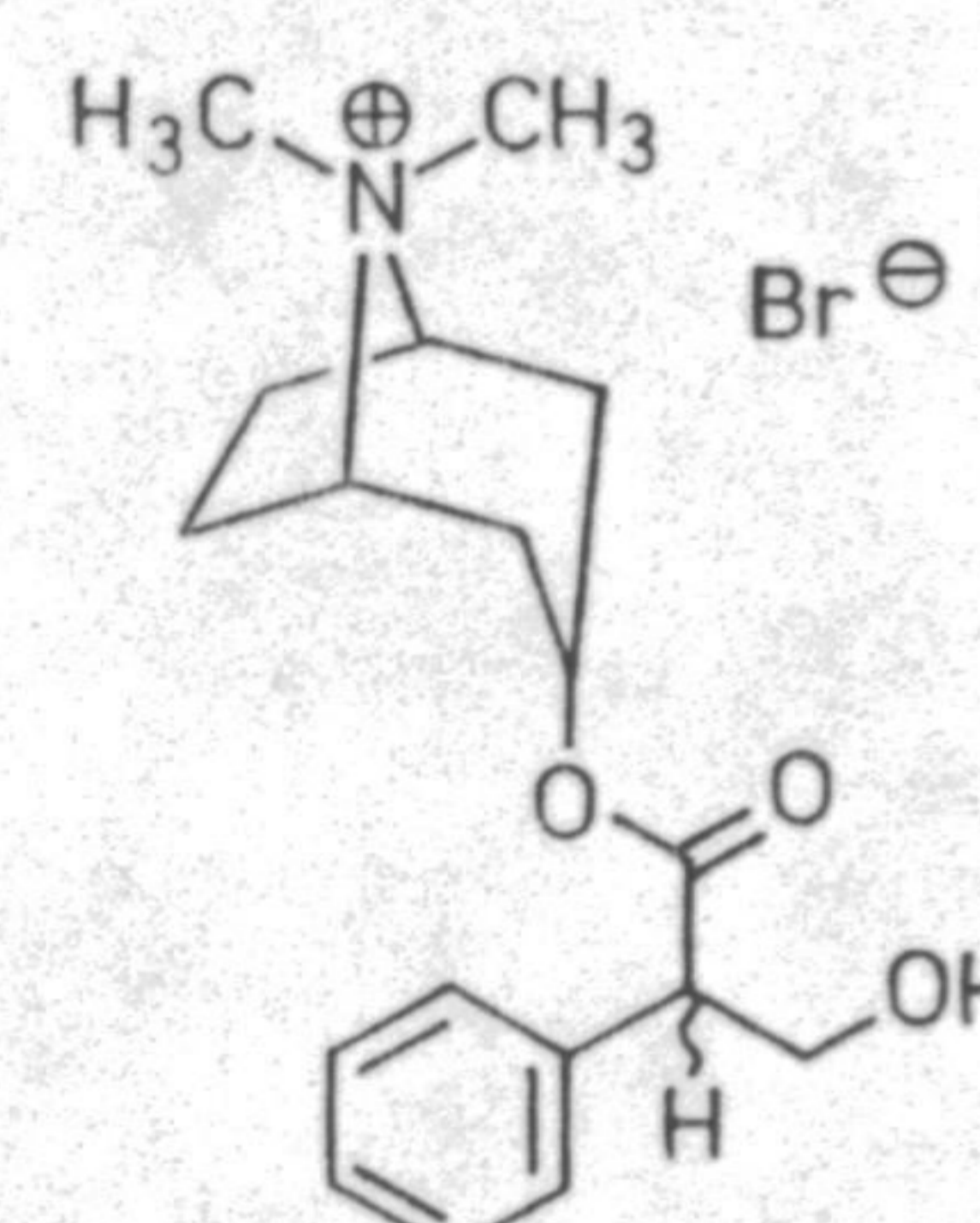
reasonable mechanistic hypothesis. Arecoline (**44**) has only shown about 50% inhibition at 50 $\mu\text{g/ml}$, and the hemisynthetic atropine methobromide (**45**) causes about 80% inhibition at this concentration (40).



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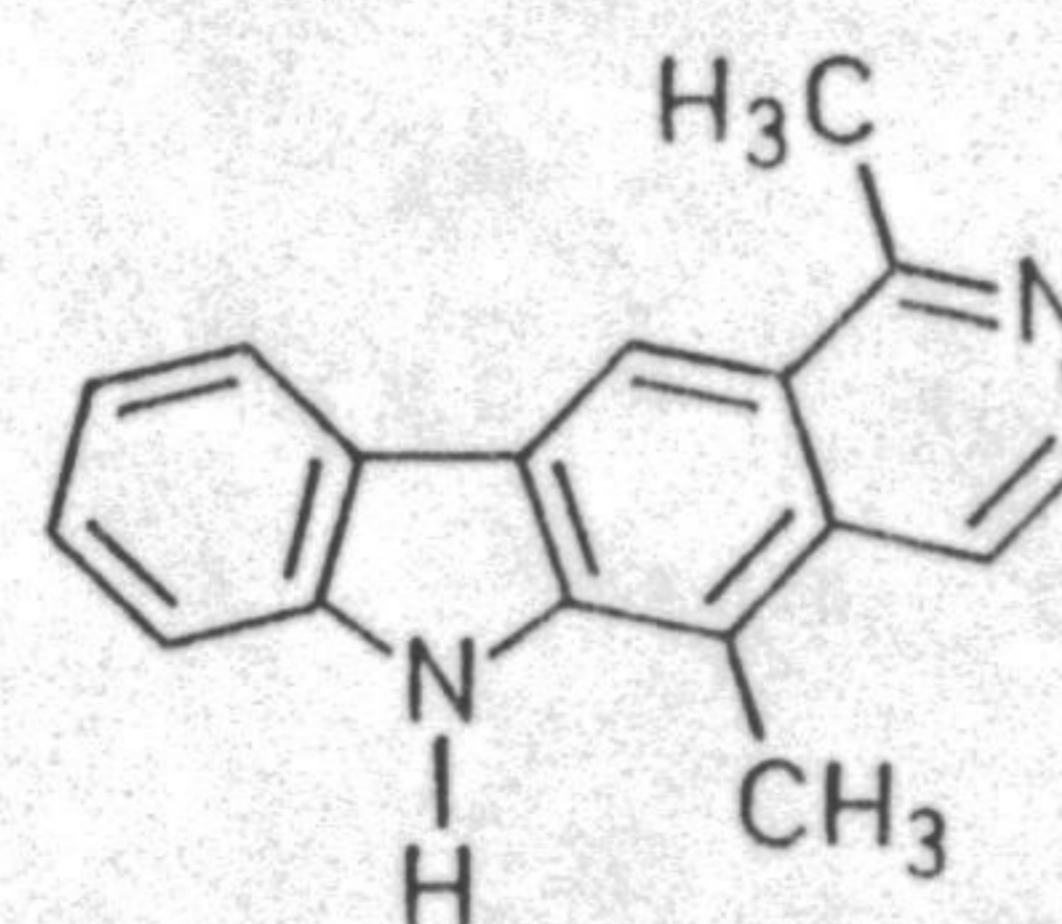


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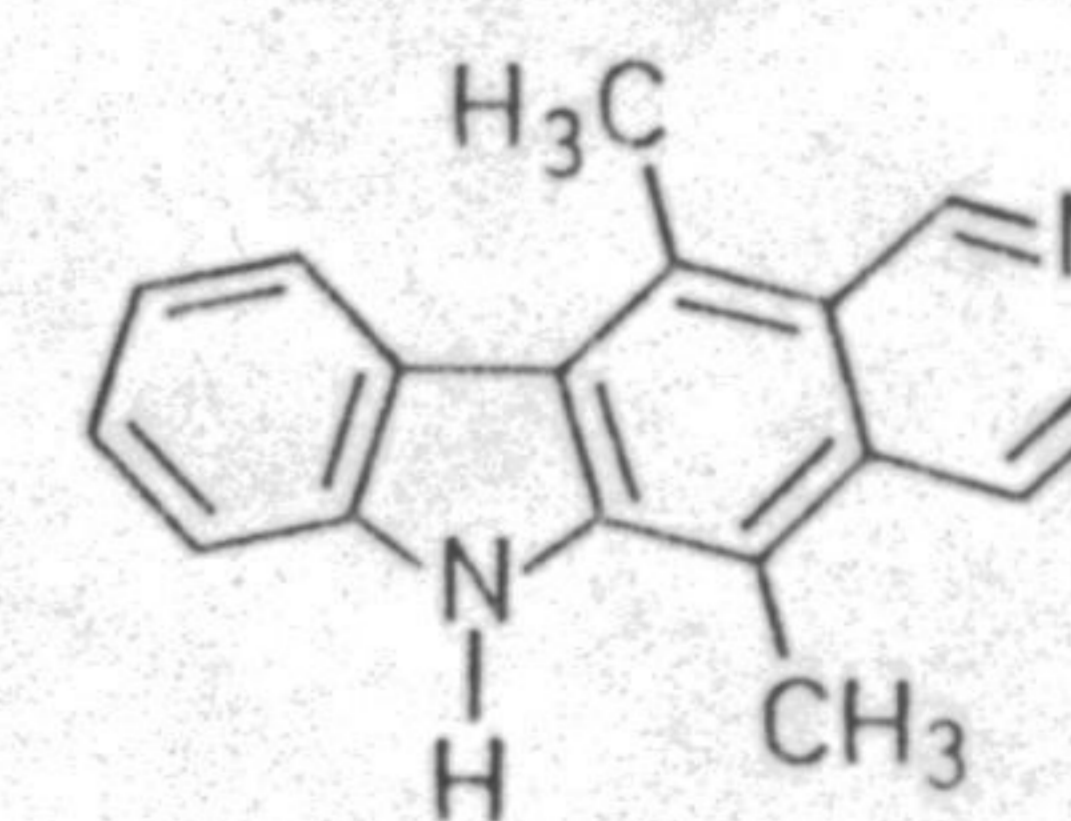


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The antitumor indole alkaloid olivacine (**46**) causes ultrastructural and metabolic alterations in *T. cruzi* epimastigotes *in vitro*, with an IC_{50} between 2.5 and 5.0 $\mu\text{g/ml}$, but failed to protect mice from infection with trypomastigotes, suggesting metabolic inactivation by the host (52). It was found that olivacine inhibits respiration and pyruvate oxidation, DNA, RNA, and protein synthesis in the parasite, with the latter effect apparently being the most significant. The olivacine isomer ellipticine (**47**) and some of its derivatives, which owe their well-known antineoplastic activity to their effects on DNA topoisomerases, also inhibit *T. cruzi* epimastigote proliferation at low concentrations (53), an effect which has been ascribed to the selective action of these products on kinetoplast topoisomerase II (54).

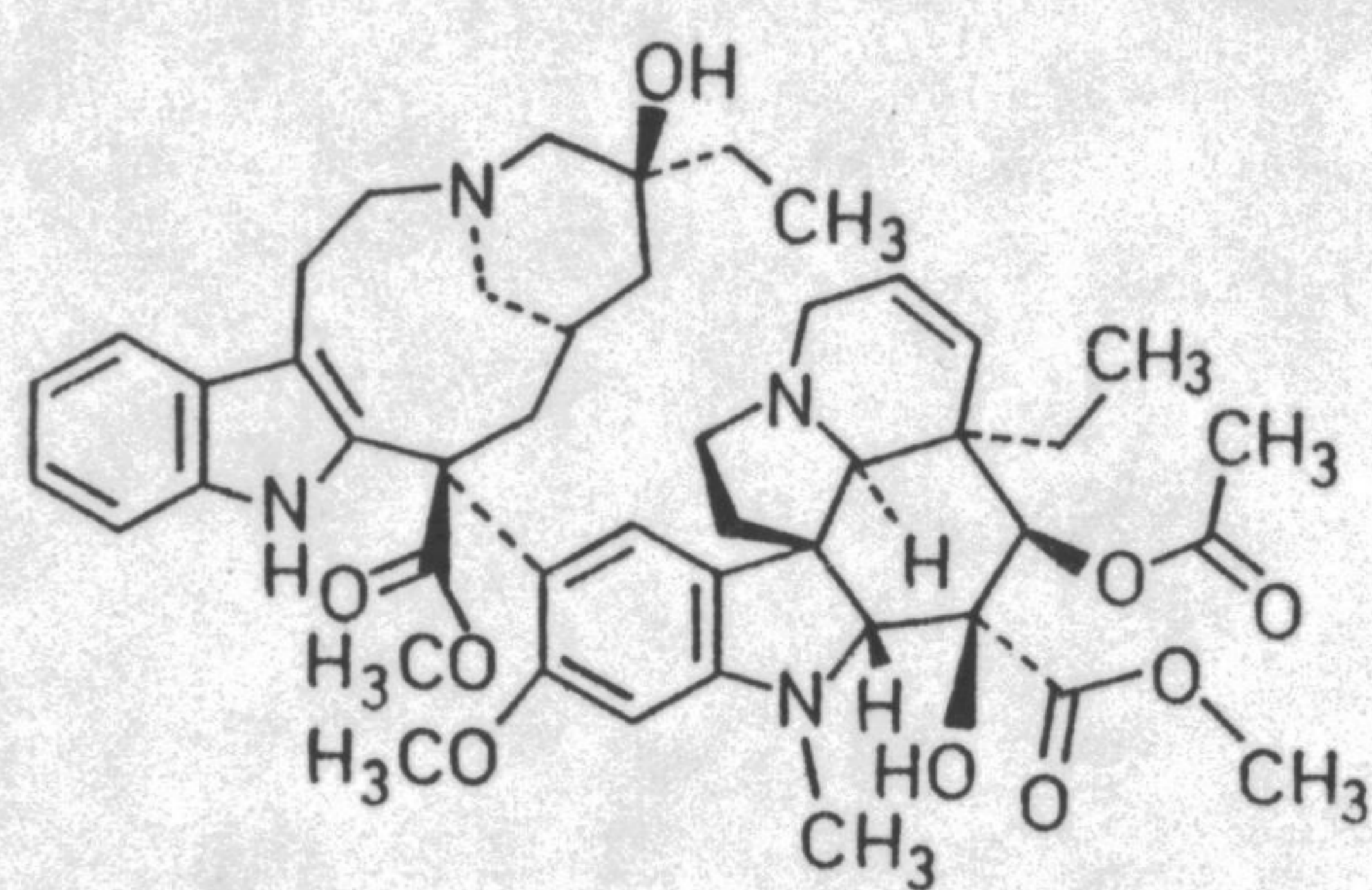


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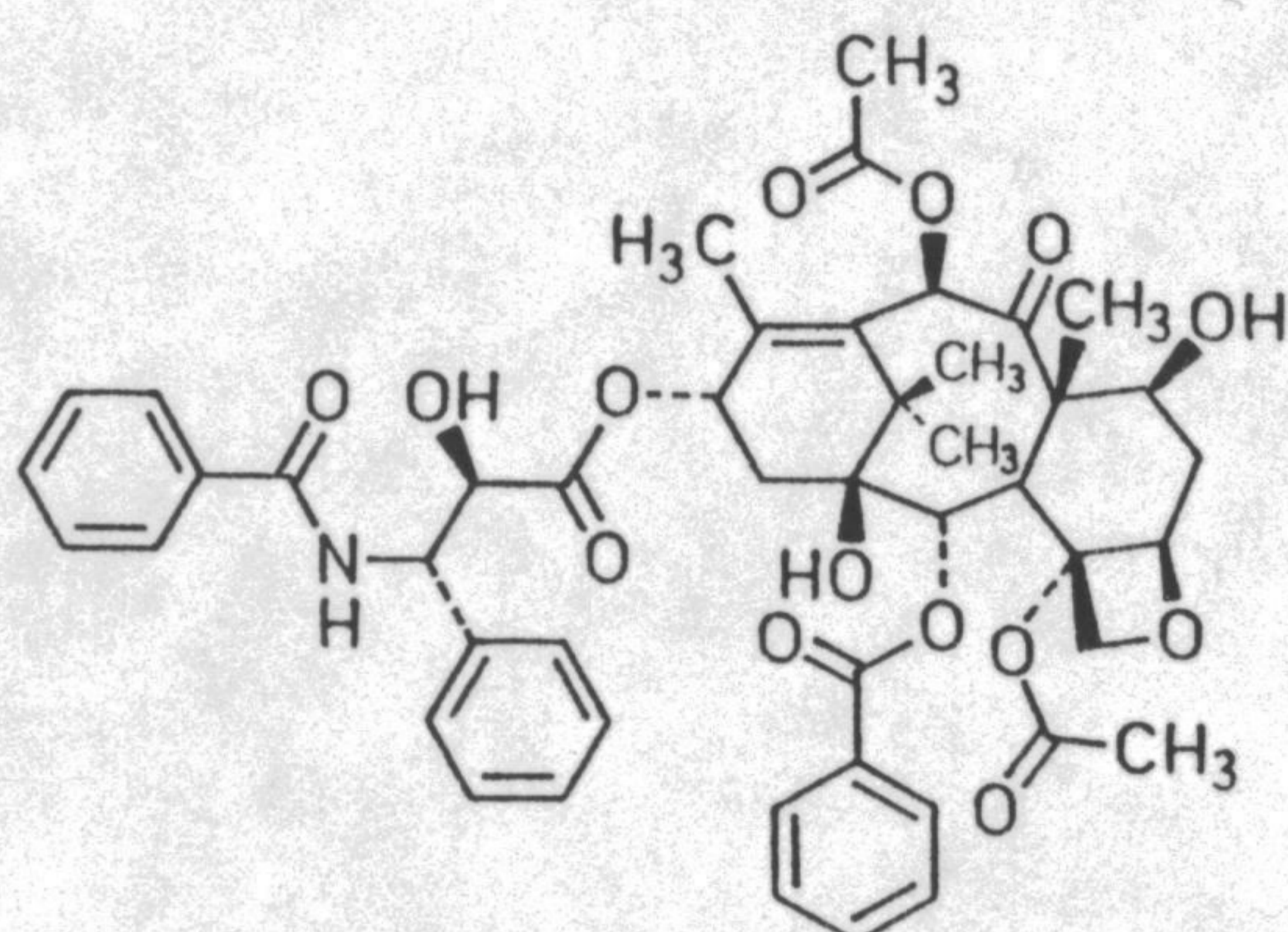


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The tubulin polymerization inhibitory antitumor alkaloid vinblastine (**48**) completely blocks *T. cruzi* epimastigote replication *in vitro* at 5 $\mu\text{g/ml}$ ($6 \mu\text{M}$) (40). The mechanistically unusual antitumor mixed biogenesis product taxol (**49**), which inhibits the depolymerization of tubulin and thus stabilizes the cytoskeleton rather than destabilizing it, may owe its ability to interfere with *T. cruzi* epimastigote replication to the same mechanism, acting at concentrations as low as 0.1 μM and with complete arrest of growth at 10 μM (55).



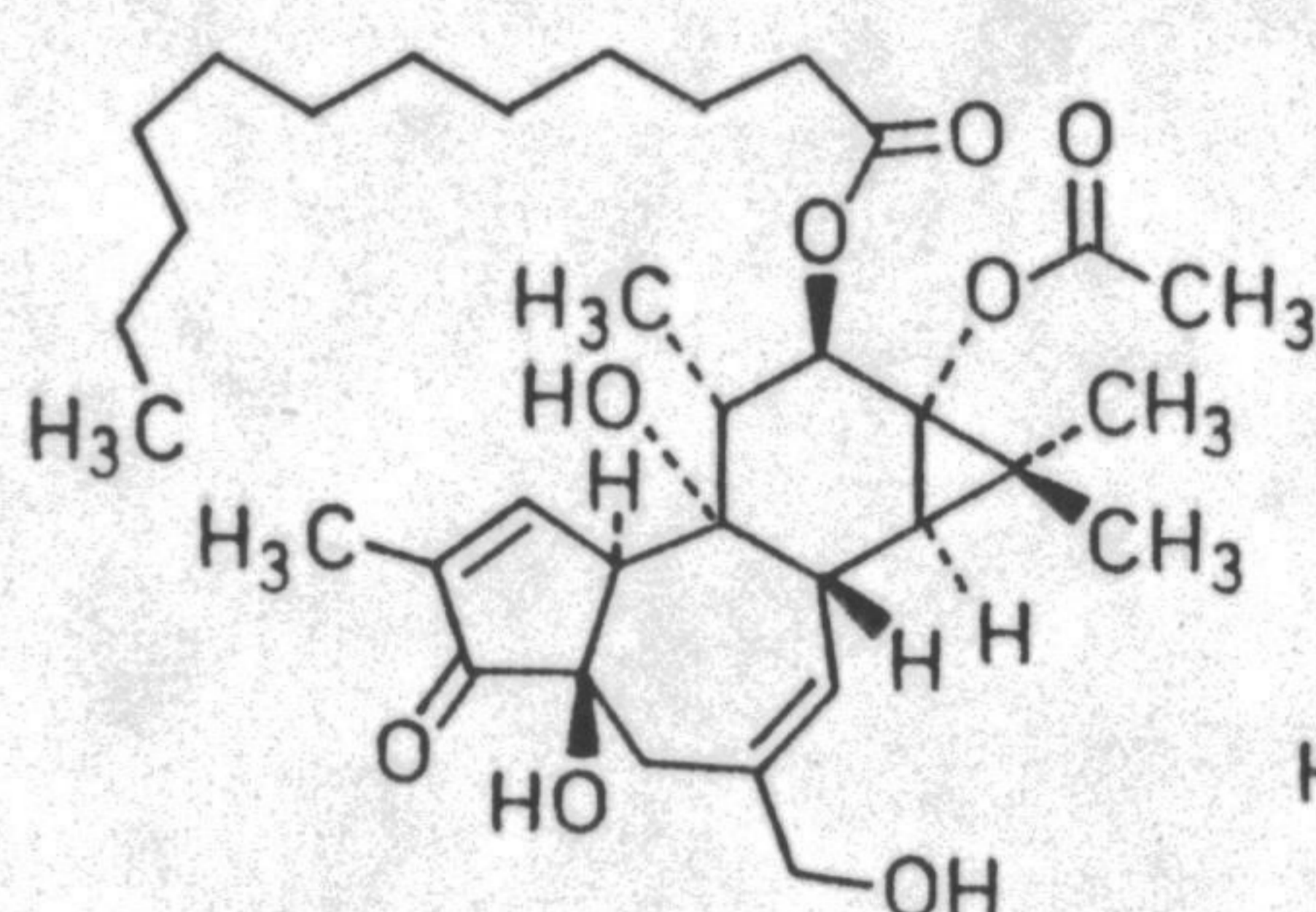
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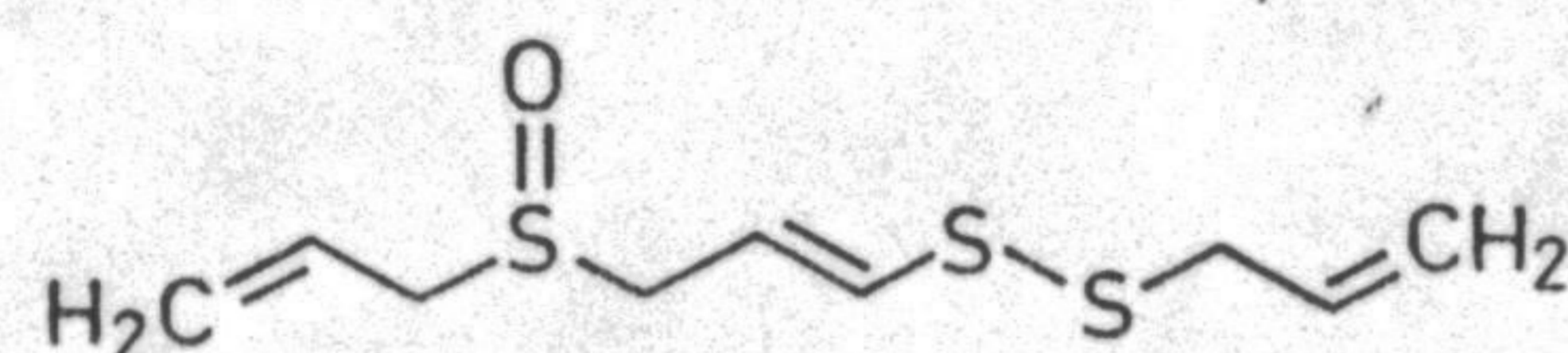
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12-*O*-Tetradecanoyl phorbol-13-acetate (**50**), possibly the best known protein kinase C-activating diterpene phorbol ester, is toxic to *T. cruzi* at concentrations as low as 0.1 $\mu\text{g/ml}$, but to a lesser extent than to related *Leishmania* species (56). As phorbol esters in general are very toxic to mammals, this compound does not seem to be a particularly attractive candidate for further development.

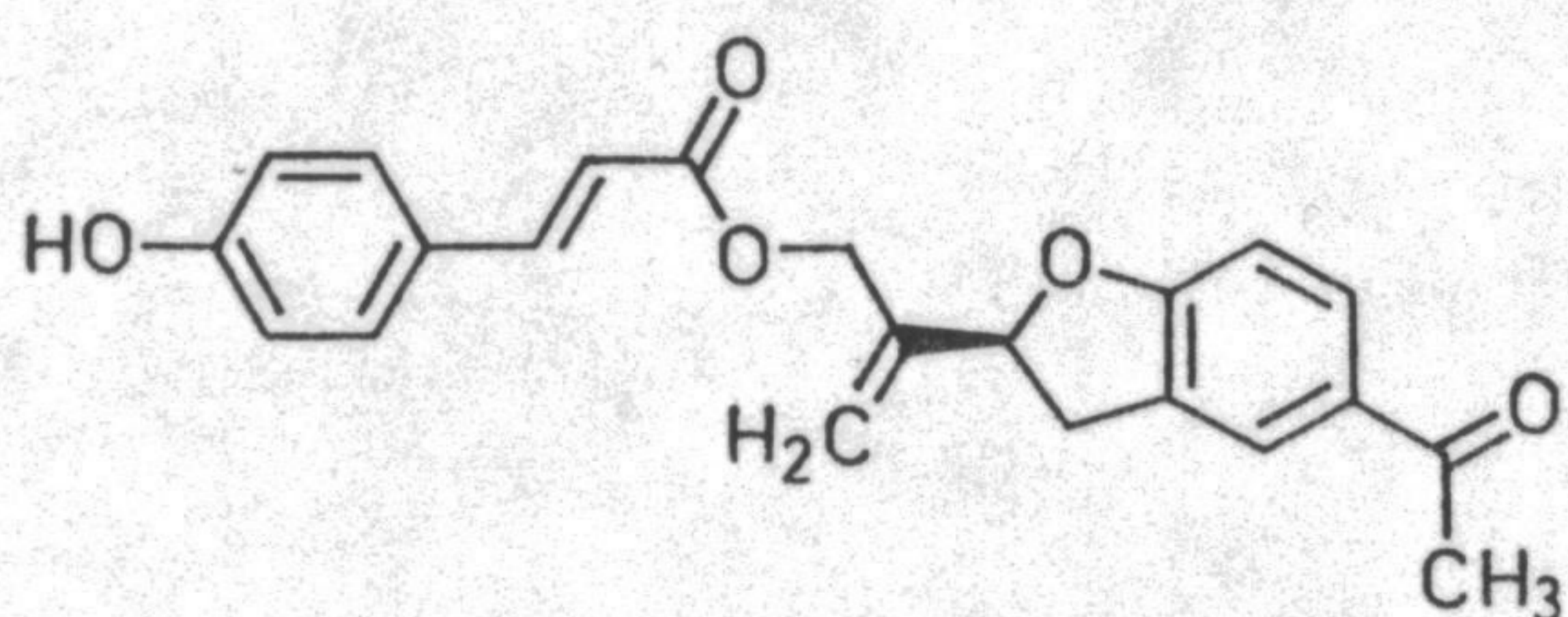
The garlic constituent ajoene [(*E,Z*)-4,5,9-trithiododeca-1,6,11-triene 9-oxide, **51**], a cysteine metabolite well known for its inhibition of platelet aggregation (57), rapidly arrests the proliferation of epimastigotes at 80 μM (20 $\mu\text{g/ml}$) and, much more significantly from the therapeutic viewpoint, is able to eradicate *T. cruzi* amastigotes growing in VERO cells within four days when added to the medium at 40 μM (10 $\mu\text{g/ml}$). This activity has been related to severe alterations in the lipid composition of the parasite, especially a shift from phosphatidylcholine as the major constituent to a predominance of phosphatidylethanolamine (58).



50



51



52

p-Coumaroyloxymetone (**52**) has a consistent marked lytic effect on trypomastigotes at concentrations as low as 20 μM (5 $\mu\text{g/ml}$) (59), but the potent neurotoxicity of tremetone (60), and the very strong hypotensive activity of the closely related dihydroeuparin (61), make the clinical use of esters of these 5-acetylbenzofuran derivatives appear unlikely for the prevention or treatment of Chagas' disease. At this point, nothing can be said about the possible mechanism of action of this product.

Conclusions

In summary, natural products active against *T. cruzi* exhibit activity at concentrations ranging from less than 1 $\mu\text{g/ml}$ to more than 1 mg/ml. They belong to a broad spectrum of chemical classes and may act by a variety of mechanisms, although in most cases these have not been clearly demonstrated. Some of these substances are antineoplastic and known for their cytotoxicity, which makes them interesting for the chemotherapy of certain cancers but possibly less attractive as selective protozoal toxicants for use in parasitic diseases due to their potential aggressiveness towards the human host. Although trypanosomal trans-sialidase may prove to be a uniquely useful target in the development of new drugs (6, 62), no natural products have yet been shown to inhibit this enzyme. Similarly, the major cysteinyl proteinase of the parasite (cruzipain), another possible target (63), is not known to be blocked by any natural product. On the other hand, a trend which emerges considering the structures and mechanisms of action reviewed above suggests that many natural products may be trypanocidal by virtue of their interference with the redox balance of the parasites, acting either on the respiratory chain or on the cellular defenses against oxidative stress. In this connection, substances specifically catalyzing redox cycling in trypanosomatids and thus increasing oxidative stress by unchecked production of superoxide anion radical may be particularly valuable leads.

A drawback of natural product screening apparent in reviews such as this is the lack of systematic structural variation which militates against any attempt at structure-activity analysis. In some cases, as with 1,2-naphthoquinones related to β -lapachone (31), limited series of synthetic products have been tested and compared with natural substances of known potency. Unfortunately, this is the exception rather than the rule. On occasion the authors of screening papers have attempted to extend their series by simple transformations such as *O*-acetylation, but acetate esters are not only less water-soluble than their precursor alcohols or phenols, and thus more difficult to administer, but also rather generally susceptible to the action of ubiquitous esterases, and therefore trivial from a pharmacological viewpoint. Finally, the almost universal use of *T. cruzi* epimastigote cultures as a biological screen, while useful to identify active compounds, neglects the different sensitivity of the infective trypomastigotes and the complications arising from the chronic disease caused by intracellular amastigotes. Moreover, the dearth of systematic biochemical (e.g. enzyme inhibition) studies on the effects of series of trypanocidal compounds presumably acting by the same mechanism continues to postpone the development of more selective drugs. It therefore seems more than reasonable that relatively potent antitrypanosomal substances (with IC_{50} below 50 $\mu\text{g/ml}$, for example, in the same potency range or more active than nifurtimox or benznidazole), particularly if

they do not belong to classes of compounds which commonly exhibit cytotoxicity, should be subjected to continued studies as to their mechanisms of action and structure-activity relationships.

The insidious character of Chagas' disease led to its going unrecognized as a separate entity by native American cultures and by Western medicine until less than a century ago. It may be thought, therefore, that ethnopharmacology has nothing to offer with regard to this problem. Nevertheless, as can be seen in a number of cases, active constituents of native remedies for cutaneous or mucocutaneous leishmaniasis caused by parasites phylogenetically and biochemically related to *T. cruzi* are also toxic to this organism. It thus makes sense to identify plants used traditionally in the treatment of the easily recognized leishmaniasis and extend screening, bioassay-guided fractionation, and isolation of the active products to their potential activity against *T. cruzi*. An integrated approach in this direction might therefore uncover important lead compounds for the development of improved drugs which are sorely needed to combat the leishmaniasis and trypanosomiasis in general.

References

- Brener, Z. (1973) *Annu. Rev. Microbiol.* 27, 347–382.
- Wendel, S., Brener, Z., Camargo, M. E., Raissi, A. (1992) Chagas disease (American Trypanosomiasis): its impact on transfusion and clinical medicine, ISBT-Sociedade Brasileira de Hematologia e Hemoterapia, São Paulo, Brazil.
- Kirchhoff, L. V. (1993) *New Engl. J. Med.* 329, 639–644.
- Zingales, B., Carniol, C., de Lederkremer, R. M., Colli, W. (1987) *Mol. Biochem. Parasitol.* 26, 135–144.
- Pereira, M. E. A. (1983) *Science* 219, 1444–1446.
- Schenkman, S., Jiang, M.-S., Hart, G. W., Nussenzweig, V. (1991) *Cell* 65, 1117–1125.
- Dias, J. C. P., Brener, Z. (1984) *Mem. Inst. Oswaldo Cruz* 79, Suppl., 139–147.
- Nussenzweig, V., Sontag, R., Biancalana, A., Freitas, J. L. P., Amato Neto, V., Kloetzel, J. (1953) *O Hospital* 44, 731–744.
- Rezende, J. M., Zupelli, V., Bufutto, M. G. (1965) *Rev. Goiana Med.* 11, 35–47.
- Letelier, M. E., Rodríguez, E., Wallace, A., Lorca, M., Repetto, Y., Morello, A., Aldunate, J. (1990) *Exper. Parasitol.* 71, 357–363.
- Morello, A., Lipchenca, I., Cassels, B. K., Speisky, H., Aldunate, J., Repetto, Y. (1994) *Comp. Biochem. Physiol.* 107C, 367–371.
- Webster, L. T., Jr., (1990) in: *Goodman and Gilman's – The Pharmacological Basis of Therapeutics*, (Goodman, A., Rall, T. W., Nies, A. S., Taylor, R., eds.) 8th edn., Macmillan, New York.
- Aldunate, J., Morello, A. (1993) Free radicals in the mode of action of antiparasitic drugs, in: *Free Radicals in Tropical Diseases*, (Aruoma, O. I., ed.), Harwood, New York.
- Brown, J. R. (1987) in: *Chemotherapy of Tropical Diseases* (Hooper, M., ed.), Wiley, New York.
- Penketh, P. G., Kennedy, W. P. K., Patton, C. L., Sartorelli, A. C. (1987) *FEBS Lett.* 221, 427–431.
- Morello, A. (1988) *Comp. Biochem. Physiol.* 90C, 1–12.
- Shames, S. L., Fairlamb, A. H., Cerami, A., Walsh, C. T. (1986) *Biochemistry* 25, 3519–3526.
- Moreno, S. N., Carnieri, E. G., Docampo, R. (1994) *Mol. Biochem. Parasitol.* 67, 313–320.
- Jockers-Scherübl, M. C., Schirmer, R. H., Krauth-Siegel, R. L. (1992) *Eur. J. Biochem.* 180, 267–272.
- Chiari, E., Braga de Oliveira, A., Raslan, D. S., Mesquita, A. A. L., Tavares, K. G. (1991) *Trans. Roy. Soc. Trop. Med. Hyg.* 85, 372–374.
- Goijman, S. G., Turrens, J. F., Marini-Bettòlo, G. B., Stoppani, A. O. M. (1984) *Medicina (Buenos Aires)* 44, 361–370.
- Hocquemiller, T., Cortes, D., Arango, G. J., Myint, S. H., Cavé, A., Angelo, A., Muñoz, V., Fournet, A. (1991) *J. Nat. Prod.* 54, 450–452.
- Castro, C., Jiménez, M., González-De La Parra, M. (1992) *Planta Med.* 58, 281–282.
- Fournet, A., Angelo, A., Muñoz, V., Roblot, F., Hocquemiller, R., Cavé, A. (1992) *J. Ethnopharmacol.* 37, 159–164.
- Docampo, R., Moreno, S. N. J. (1984) in: *Free Radicals in Biology*, Academic Press, London, pp. 243–288.
- Hazra, B., Sur, P., Sur, B., Banerjee, A., Roy, D. K. (1984) *Planta Med.* 51, 295–298.
- Docampo, R., Cruz, F. S., Boveris, A., Muñoz, R. P. A., Esquivel, D. M. S. (1978) *Arch. Biochem. Biophys.* 186, 292–297.
- Lopes, J. N., Cruz, F. S., Docampo, R., Vasconcellos, M. E., Sampaio, M. C. R., Pinto, A. V., Gilbert, B. (1978) *Ann. Trop. Med. Parasitol.* 72, 523–531.
- Gonçalves, A. M., Vasconcellos, M. E., Docampo, R., Cruz, F. S., De Souza, W., León, W. (1980) *Mol. Biochem. Parasitol.* 1, 167–176.
- Boveris, A., Sies, H., Martino, E. E., Docampo, R., Turrens, J. F., Stoppani, A. O. M. (1980) *Biochem. J.* 188, 643–648.
- Molina Portela, M. P., de Pahny, E. M., Galeffi, C., Stoppani, A. O. M. (1991) *Rev. Arg. Microbiol.* 23, 1–14.
- Morello, A., Pavani, M., Garbarino, J. A., Chamy, M. C., Frey, C., Mancilla, J., Guerrero, A., Repetto, Y., Ferreira, J. (1995) *Comp. Biochem. Physiol.*, in press.
- Goijman, S. G., Turrens, J. F., Marini-Bettòlo, G. B., Stoppani, A. O. M. (1985) *Experientia* 41, 646–648.
- Goijman, S. G., Turrens, J. F., Marini-Bettòlo, G. B., Stoppani, A. O. M. (1984) *Medicina (Buenos Aires)* 44, 361–370.
- Campanelli, A. R., D'Alagni, M., Marini-Bettòlo, G. B. (1980) *FEBS Lett.* 122, 256–259.
- Fournet, A., Muñoz, V., Roblot, F., Hocquemiller, R., Cavé, A., Gantier, J.-C. (1993) *Phytotherapy Res.* 7, 111–115.
- Blanco, A., Aoki, A., Montamat, E. E., Rovai, L. E. (1983) *J. Protozool.* 30, 648–651.
- González-Garza, M. T., Said-Fernández, S. (1988) *Exp. Parasitol.* 66, 253–255.
- Rovai, L. E., Aoki, A., Gerez de Burgos, N. M., Blanco, A. (1983) *J. Protozool.* 30, 648–651.
- Cavin, J. C., Krassner, S. M., Rodríguez, E. (1987) *J. Ethnopharmacol.* 19, 89–94.
- Evans, A. T., Croft, S. L. (1987) *Phytother. Res.* 1, 25–27.
- Buckholtz, N. S., Boggan, W. O. (1977) *Biochem. Pharmacol.* 26, 1991–1996.
- Canessa, M., Jaimovich, E., De la Fuente, M. (1973) *J. Membrane Biol.* 13, 263–282.
- Lea, T. J., Ashley, C. C. (1981) *Biochim. Biophys. Acta* 664, 74–81.
- Naranjo, C. (1969) in: *Ethnopharmacologic Search for Psychoactive Drugs*, (Efron, D. H., Holmstedt, B., Kline, N. S., eds.), Raven, New York.
- Rivas, P., Cassels, B. K., Morello, A., Repetto, Y. (1995) unpublished results.
- Ammann, M., Nagakura, N., Zenk, M. H. (1984) *Tetrahedron Lett.* 25, 953–954.
- Fournet, A., Manjon, A. M., Muñoz, V., Angelo, A., Bruneton, J., Hocquemiller, R., Cortes, D., Cavé, A. (1988) *J. Ethnopharmacol.* 24, 337–344.
- Cassels, B. K., Asencio, M., Conget, P., Speisky, H., Videla, L. A., Lissi, E. A. (1995) *Pharmacological Research* 31, 103–107.
- Mahiou, V., Roblot, F., Hocquemiller, R., Cavé, A., Rojas de Arias, A., Inchausti, A., Yaluff, G., Fournet, A., Angelo, A. (1994) *J. Nat. Prod.* 57, 890–895.
- Fournet, A., Hocquemiller, R., Roblot, F., Cavé, A., Richomme, P., Bruneton, J. (1993) *J. Nat. Prod.* 56, 1547–1552.
- Leon, L., Vasconcellos, E. D., Leon, W., Cruz, F., Docampo, R., De Souza, W. (1978) *Exper. Parasitol.* 45, 151–159.
- Douc-Rasy, S., Kayser, A., Riou, G. (1983) *Biochem. Biophys. Res. Commun.* 117, 1–5.

- ⁵⁴ Douc-Rasy, S., Kayser, A., Riou, J. F., Riou, G. (1986) Proc. Natl. Acad. Sci. USA 83, 7152–7156.
- ⁵⁵ Baum, S. G., Wittner, M., Madler, J. P., Horowitz, S. B., Schiff, P. B., Tanowitz, H. B. (1981) Proc. Natl. Acad. Sci. USA 78, 4571–4575.
- ⁵⁶ Vannier-Santos, M. A., Pimenta, P. F. P., De Souza, W. (1988) J. Submicrosc. Cytol. Pathol. 20, 583–593.
- ⁵⁷ Apitz-Castro, R., Jain, M. K., Bartoli, F., Ledezma, E., Ruiz, M. C., Salas, R. (1991) Biochim. Biophys. Acta 1095, 269–280.
- ⁵⁸ Urbina, J. A., Marchan, E., Lazard, K., Visbal, G., Apitz-Castro, R., Gil, F., Aguirre, T., Piras, M. M., Piras, R. (1993) Biochem. Pharmacol. 45, 2381–2387.
- ⁵⁹ González, J., Sagua, H., Araya, J., Loyola, A., Morales, G., Pereira, J., Estrada, M. (1990) Phytother. Res. 4, 1–4.
- ⁶⁰ Bonner, DeGraw (1962) Tetrahedron 18, 1295.
- ⁶¹ Cerda, C. J. (1986) Dissertation (Pharmacy), University of Chile.
- ⁶² Colli, W. (1993) FASEB J. 7, 1257–1264.
- ⁶³ Meirelles, M. N. L., Juliano, L., Carmona, E., Silva, S. G., Costa, E. M., Murta, A. C. M., Scharfstein, J. (1992) Mol. Biochem. Parasitol. 52, 175–184.