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THE PREPARATION AND UTILIZATION OF PARAHERQUAMIDE-2-O-METHYL IMIDATE IN THE SYNTHESIS OF 14-O-SUBSTITUTED PARAHERQUAMIDE DERIVATIVES

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Abstract. The preparation of the methyl imidate of the antiparasitic oxindole alkaloid paraherquamide, its utilization in the synthesis of a variety of 14-O-substituted paraherquamide analogs and their biological activity are described.

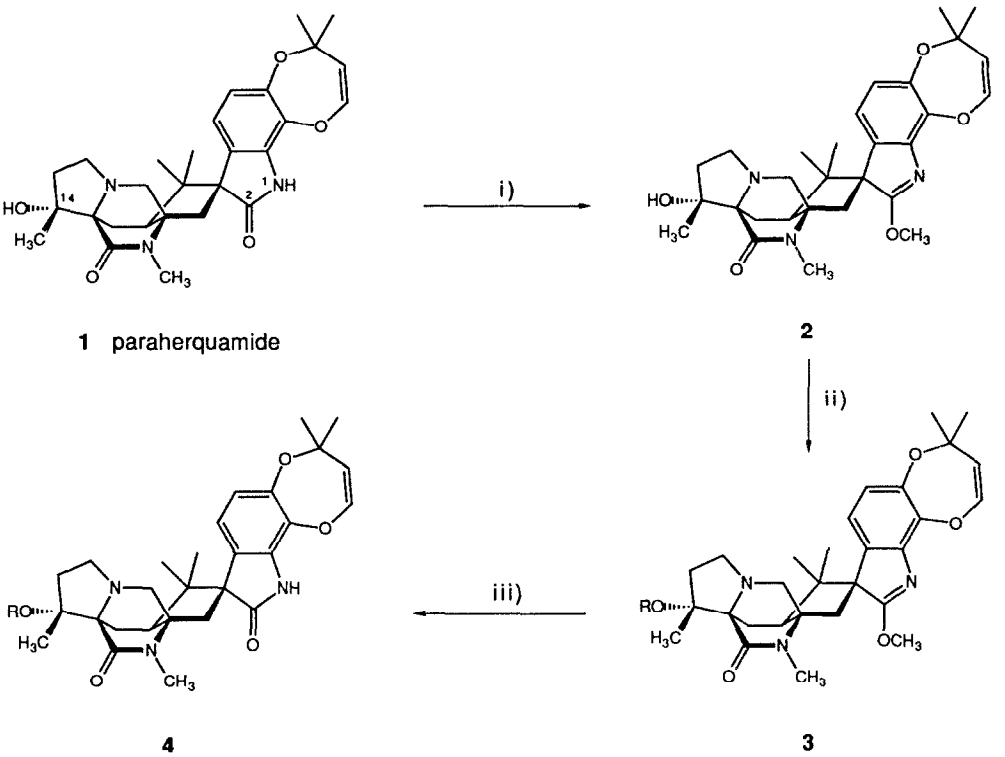
In an antiparasitic screening program at Merck, a culture of *Penicillium charlesii* was found that possessed anthelmintic activity in gerbils.[1] The active component was subsequently purified and identified as paraherquamide **1**,[2] a novel oxindole alkaloid first isolated and characterized in 1981 by Yamazaki and co-workers from culture broths of *Penicillium paraherquei*.[3] Further testing demonstrated that paraherquamide is also a potent broad spectrum anthelmintic in sheep.[4] Although the mode of action of paraherquamide has not been determined, it is thought to be unrelated to that of ivermectin or of thiabendazole since it is equally effective against both ivermectin- and thiabendazole-sensitive and -resistant helminths.[4] Paraherquamide has been shown to specifically bind to membranes obtained from the free living nematode *Caenorhabditis elegans*.[5] The anthelmintic compound phenothiazine and several phenothiazine analogs are competitive inhibitors of [^{3}H] paraherquamide binding suggesting that the compounds have a common or related binding site.[5] The potent anthelmintic activity of paraherquamide makes this compound the object of much interest.

A synthetic chemical program was initiated to elucidate the structural features required for activity and to improve the compound's therapeutic index. Previous publications have described some of the interesting chemistry carried out toward this goal.[6] Early in these investigations it was discovered that the 14-O-trimethylsilyl ether of paraherquamide **4a** was nearly equipotent with the parent compound in the *C. elegans* motility assay.[6b] In order to determine whether the silyl ether was acting as a prodrug or whether the compound was intrinsically active, the synthesis of a variety of 14-O-substituted paraherquamide derivatives was undertaken. As might be expected, reaction of paraherquamide with base and an electrophile led to alkylation at the secondary amide nitrogen and, in most cases, resulted in a significant loss of activity.[6a] Initial attempts to prepare and alkylate the 1,14-dianion of the alkaloid were unsuccessful.[6a] In order to prepare the desired ethers, an appropriately protected form of paraherquamide was required. Protection of the secondary amide as the corresponding methyl imidate **2** was considered the most suitable approach.

Initial efforts to prepare paraherquamide-2-O-methylimidate using trimethyloxonium- or triethyloxonium tetrafluoroborate did not yield the desired product. The silica gel mediated methylation of amides with diazomethane to afford the corresponding imidates has been reported[7] and this methodology was explored. Thus, a dichloromethane solution of paraherquamide was treated with an excess of ethereal diazomethane in the presence of silica gel. Although produced in modest yield (40%) the desired methyl imidate **2** was obtained as the major product. The imidate was stable to a variety of basic and nucleophilic reagents (NaH, $NaNH_2$,

$LiAlH_4$, $NaBH_4$, CH_3MgBr), but was readily hydrolyzed under mildly acidic conditions. Accordingly, treatment of **2** with 4 equivalents of 1N aqueous HCl in THF at room temperature resulted in regeneration of the amide group.

Alkylation of **2** was carried out by treatment of the imidate with sodium hydride (5-8 eq.) and the electrophile (5-8 eq.) in either THF (entries g and h) or DMF. The alkylation, a straightforward Williamson ether synthesis, worked best for small or activated electrophiles (methyl iodide, benzyl bromide, allyl iodide). Yields fell off with simple primary halides (iodoethane, iodopropane). Treatment of **2** with propargyl bromide led to the formation of the expected propargylic ether **4d** along with the unstable allenic ether. Use of methyl isocyanate as the electrophile resulted in formation of the ureidocarbonyl species **4g**. Also, employing MEM-Cl as the alkylating agent afforded 14-O-(methoxyethoxymethyl)paraherquamide **4f** directly, without isolation of intermediate **3f**.



1 paraherquamide **2** **3** **4**

i) CH_2N_2/Et_2O, SiO_2, CH_2Cl_2. ii) NaH, R-X, DMF. iii) H_3O^+, THF.

The use of an assay measuring the inhibition of motility of the nematode *C. elegans* has been reported as a primary screen for anthelmintic compounds.[8] As shown in Table 1, a number of the 14-O-substituted paraherquamide derivatives showed good activity in the *C. elegans* motility assay. The smaller alkyl ethers (ethyl **4b**, butyl **4c**, and propargyl **4d**) were nearly as potent as paraherquamide. The allyl- and methoxyethoxymethyl-ether derivatives **4e** and **4f** were somewhat less active while attachment of bulkier groups

to the alkaloid led to a significant loss of activity. The noteworthy exception to this trend was the methyl ether of paraherquamide **4i**, which was much less active than either the parent or ethyl analog. The reason for the surprisingly low activity of the methyl ether is not yet clear.

TABLE 1: PARAHERQUAMIDE DERIVATIVES: YIELDS AND *IN VITRO* ACTIVITY[9]

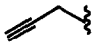
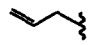
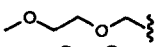
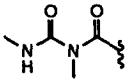
ENTRY	ELECTROPHILE	R	YIELD 3	YIELD 4	*C. elegans* motility assay IC_{50} μg/ml
Paraherquamide		H—	--	--	2.5
a	----	TMS—	--	--	3
b	iodoethane		40	75	2
c	iodobutane		19	60	5
d	propargyl bromide		11	44	7.5
e	allyl bromide		64	72	15
f	MEM-Cl		--	34	20
g	methyl isocyanate		42	57	60
h	benzyl bromide		46	68	65
i	iodomethane	H_3C—	72	62	70

TABLE 2: ANTHELMINTIC ACTIVITY OF 14-0 ALLYLPARAHERQUAMIDE IN SHEEP[a]

Compound	Dosage mg/kg	*H.c.*	*Os.c.*	*T.a.*	*T.c.*	*C.spp.*	*Oe.c.*[b]
1	2.0	3	3	3	3	3	2
1	0.5	3	3	3	3	3	0
4e	2.0	3	3	3	3	3	1
4e	0.5	3	3	3	3	3	0

(a) efficacy as % reduction from control: 0 = <50%, 1 = 51-75%, 2 = 76-90%, 3 = 91-100%; (b) *H.c.* = *Haemonchus contortus*, *Os.c.* = *Ostertagia circumcincta*, *T.a.* = *Trichostrongylus axei*, T.c. = *Trichostrongylus colubriformus*, *C.spp* = *Cooperia species*, *Oe. c.* = *Oesophagostomum columbianum.*

14-O-Allyl-paraherquamide **4e** was tested in sheep experimentally infected with six nematodes (Table 2). The sheep were given a single oral dose of **4e** at levels of either 2.0 or 0.5 mg/kg. One week after treatment the sheep were necropsied and examined for residual worm burdens compared to untreated infected controls. At

both doses compound **4e** was effective against five of the six parasites. The drug had only weak activity against *Oesophagostomum columbianum*. This spectrum of activity is comparable to that of the paraherquamide itself.[5]

In summary, this communication reports the preparation of paraherquamide-2-O-methyl imidate and its utility as an intermediate in the synthesis of 14-O-alkyl derivatives of paraherquamide. The compounds disclosed maintain significant bioactivity as demonstrated by their efficacy in the *C. elegans* motility assay, and the allyl ether of paraherquamide has been shown to be a potent anthelmintic in sheep. Synthesis and activity of other novel analogs of paraherquamide will be forthcoming.

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