Journal of Natural Products
Vol. 49, No. 3, pp. 530-533, May-Jun 1986

THE EFFECT OF NATURALLY OCCURRING NAPHTHOQUINONES ON VELVETLEAF (*ABUTILON THEOPHRASTI*) GERMINATION

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Juglone (**1c**) is a classic example of an allelopathic chemical (1,2). Many other naphthoquinones are known to occur in nature (3), but as yet, only juglone is considered to be an "inhibitor produced by higher plants which is definitely known to be a naphthoquinone" (4). In the course of our study of plant constituents showing weed control potential (5), we observed that plumbagin (**1h**) from an extract of *Plumbago europaea* L. inhibited the germination of velvetleaf (*Abutilon theophrasti* Medic.) seeds. This observation, coupled with the known biological effects of naphthoquinone derivatives (6), initiated this study into the antigermination activities of other plant-produced naphthoquinones.

Unfortunately, we were unable to obtain the plant sources of many known compounds because they have Asian, African, or Australian origins (3). Several of these were synthesized together with a few others that are not known to occur in plants. Considering the historical commercial importance of natural naphthoquinones, it is surprising that their herbicidal properties have not heretofore been examined more thoroughly. The few reports include: 5-hydroxy-2-methoxynaphthoquinone from *Platycarya strobilacea* Sieb et Zucc. as a lettuce germination inhibitor (7), the absence of phytotoxicity of lawsone (**1d**) when sprayed on young plants (8), and the isolation of a group of compounds inhibitory to lettuce from a shoot blight fungus (9). Several synthetic amino naphthoquinone derivatives have been evaluated as herbicides (10,11) and were indicated to have potential in sugar beets and rice.

RESULTS AND DISCUSSION

Structures of the compounds tested and their antigermination activities against velvetleaf are given in Table 1. Effective concentrations for many compounds are similar to those reported by Rietveld (12) (1 mM) for germination inhibition by juglone in a similar test involving 16 different seed species. Compounds giving 100% germination (as compared to controls) at levels of 10 mM were not tested at higher concentrations.

In general, the more highly substituted compounds had little or no activity, and longer side chains tended to decrease effectiveness. Lomatiol (**1r**) was the only compound with significant hydrocarbon substitution to show some toxicity. These results are in accord with the observation of Harborne (2) that allelochemicals tend to be rather simple molecules with low molecular weights.

EFFECT OF SUBSTITUENT.—An examination of the data from the less highly substituted compounds provides some insight into the kinds of substituents that affect activity. In most cases, one methyl group has nearly the same effect as a proton. This conclusion can be drawn from comparison of the unsubstituted compound (**1a**) with menadione (**1b**), juglone (**1c**) with plumbagin (**1h**), **1f** with **1i**, and lawsone (**1d**) with phthiocol (**1k**). A slight decrease in activity was produced by the introduction of a methyl group into the

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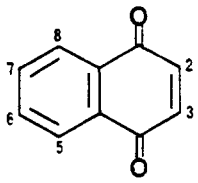
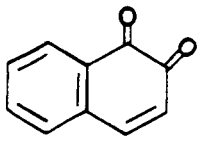
TABLE 1. Structure and Activity of Naphthoquinones



1



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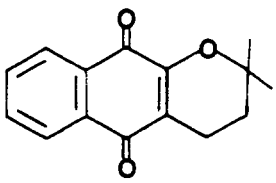

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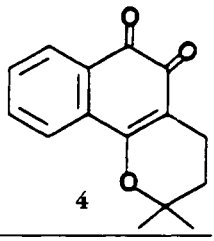

4

			Concentration mM (% germination)[a]			
1	1,4-Naphthoquinones	Substitution				
a	1,4-Naphthoquinone	none	0.6(96)	0.9(69)	1.2(28)	2.5(3)
b	Menadione	2-CH_3	0.7(92)	0.9(61)	1(47)	1.2(3)
c	Juglone	5-OH	0.4(100)	0.8(74)	1(41)	3(6)
d	Lawsone	2-OH	1.2(97)	1.8(71)	2.1(33)	2.5(10)
e	2-Methoxy	2-OCH_3	0.2(96)	0.4(70)	0.5(52)	0.6(10)
f	5-Methoxy	5-OCH_3	2(100)	3(84)	4(71)	6(5)
g	2-Acetoxy	2-$OCOCH_3$		3(92)	4(51)	5(26)
h	Plumbagin	2-CH_3 5-OH	0.2(100)	0.5(66)	0.7(22)	0.8(12)
i	2-Methyl-5-methoxy	2-CH_3 5-OCH_3	1(98)	2(58)	4(27)	5(0)
j	7-Methyljuglone	5-OH 7-CH_3		4(100)	6(45)	8(5)
k	Phthiocol	2-OH 3-CH_3	1(93)	2(68)	4(18)	6(0)
l	2-Methoxy-3-methyl	2-OCH_3 3-CH_3	0.8(92)	1(68)	2(21)	4(0)
m	Chimaphilin	2-CH_3 7-CH_3		1(90)	2(55)	4(8)
n	2,3-Dimethyl	2-CH_3 3-CH_3				10(100)
o	6,7-Dimethoxy	6-OCH_3 7-OCH_3				10(100)
p	Lapachol	2-$CH_2CH{=}C(CH_3)_2$ 3-OH			10(100)	
q	Isolapachol	2-$CH{=}CHCH(CH_3)_2$ 3-OH			10(100)	
r	Lomatiol	2-$CH_2CH{=}CCH_3CH_2OH$ 3-OH		4(97)	6(77)	8(55)
s	Vitamin K_1	2-CH_2CH-$CCH_3(CH_2CH_2CH_2CHCH_3)_3CH_3$ 3-CH_3			10(100)	
t	Naphthazarin	5-OH 8-OH			10(100)	
u	Shikonin	2-$CHOHCH_2CH{=}C(CH_3)_2$ 5-OH 8-OH			10(100)	
v	Cordeauxiaquinone	2-OH 3-CH_3 5-OH 6-CH_3 7-$COCH_3$ 8-OH			10(100)	
w	Isodiospyrin	5-OH 7-CH_3 8-**1j**			10(100)	
x	Cassumunaquinone	2-OCH_3 8-2,3-dimethoxy phenyl			10(100)	
3	α-Lapachone				10(100)	
2	1,2-Naphthoquinones					
a	1,2-Naphthoquinone	none			10(100)	
b	4-Methoxy	4-OCH_3	0.5(95)	0.8(75)	1(28)	2(5)
4	β-Lapachone				10(100)	

[a]Percent germination as compared to controls. Figures below 85 are significantly different from controls at the 95% level by the Chi-square 1-tailed test.

2-methoxy compound (**1e**) to give **1l**. But, a far greater change in toxicity occurred when additional methyl groups were added to menadione (**1b**) giving chimaphilin (**1m**) and the 2,3-dimethyl compound (**1n**). While chimaphilin was slightly less active than menadione, **1n** showed no effect upon a tenfold increase in concentration. (It is interesting to note that chimaphilin is a natural

product while **1n** is not.) Comparison of 7-methyljuglone (**1j**) with juglone shows a drastic decrease due to a methyl group, but this may involve a positional factor which will be discussed later.

Methylation of hydroxy groups yielded divergent properties. While this transformation decreased the potency of juglone (compare **1c** with **1f**), it markedly enhanced that of lawsone (**1d** vs **1e**). In the latter instance, one might deduce that the addition serves to stabilize the molecule by prohibiting tautomerism and thus preserving the active form (compare **1a** and **2a**). Acetylation (**1g**), however, failed to change the activity of lawsone. Methyl-addition to plumbagin had about the same effect as it did to juglone (see **1i** and **1f**), whereas it tended to increase the toxicity of phthiocol (compare **1k** and **1l**). We have no evidence that phthiocol tautomerizes, and, inexplicably, introduction of a methoxyl group developed activity into 1,2-naphthoquinone (**2b**).

The only dimethoxy compound tested, **1o**, is not (to our knowledge) a natural product and was included only because of its availability. The addition of a second hydroxyl group to juglone to give naphthazarin (**1t**) appeared to stifle effectiveness.

EFFECT OF POSITION.—Juglone (**1c**), the known allelopath, appears to be effective over a fairly wide range of concentrations; whereas its isomer, lawsone (**1d**), is much less active at low concentrations and seems to have a rather steep dose/response relationship. As discussed earlier, the isomeric methoxynaphthoquinones **1f** and **1g** prepared from **1c** and **1d** reverse this relationship, with the 2-isomer having far greater activity. Another set of isomers, plumbagin (**1h**) and 7-methyljuglone (**1j**) (both natural products), have vastly different toxicities. On the basis of our results, it appears that substitution at carbon 7 tends to decrease activity. Although other factors are probably involved, the two 7-substituted compounds tested require higher concentrations for inhibition than their unsubstituted counterparts. (Compare **1b** with **1m** and **1c** with **1j**). Isodiospyrin (**1w**), the dimer of 7-methyljuglone, is not only 7-substituted but also is a complex compound with a relatively high molecular weight.

Of the compounds examined here, those with natural origins tend to exhibit toxicity at lower concentrations than those with no known natural source. Exceptions to this statement include the complexly substituted natural compounds and those with longer side chains. Harborne (2) has suggested that plants might sequester protective compounds in a benign form (e.g., a glucoside) that can be released when beneficial to the plant. Conceivably, the "inactive" compounds studied here might have a release mechanism in their parent plants that would "defuse" them by shortening a side chain or removing substituents and thus produce an active agent.

It is important to point out that, so far, only juglone has been shown to be allelopathic. However, we now know that several naphthoquinones are phytotoxic, and this information implies that one or more of them may be involved in allelopathy. Proof of this supposition requires further work and is beyond the scope of this report.

EXPERIMENTAL

Bioassays were conducted and evaluated essentially as described by Wolf *et al.* (5). The compounds were made up in stock solutions of known concentrations in $CHCl_3$. An aliquot necessary to give the appropriate final concentration (in 4 ml of H_2O) was evenly spaced over a double layer of Whatman #1 filter paper in a 9-cm petri dish. The $CHCl_3$ was allowed to evaporate, 20 surface-sterilized velvetleaf seeds were evenly distributed about the dish, and 4 ml of H_2O was added. The dishes were wrapped in foil and allowed to stand at a constant temperature of 30° for 7 days. Then, germinated seeds (those with a protruded radicle) were tallied. Each treatment consisted of two dishes (40 seeds), and each concentration was run in duplicate. A control set (4 ml of H_2O only) accompanied each treatment. Results (Table 1)

represent the averages of duplicate runs and are expressed as the number of treated seeds germinated divided by the number of control seeds germinated times 100.

Spectroscopic and chromatographic equipment have been described previously (13). Menadione (**1b**), juglone (**1c**), lawsone (**1d**), plumbagin (**1h**), and vitamin K_1 (**1s**) were purchased from Sigma Chemical Co., and 1,4-naphthoquinone (**1a**), naphthazarin (**1t**), and 1,2-naphthoquinone (**2a**) from Aldrich Chemical Co. Methoxy adducts of **1c, 1d, 1h,** and **1k** were prepared as described by Tezuka *et al.* (14), and the products were isolated by hplc. Lawsone (**1d**) yielded, in addition to **1e**, an isomeric 1,2-naphthoquinone adduct (**2b**) from the tautomer (3). Acetylation of **1d** in Ac_2O-pyridine (2:1) gave **1g**. Shikonin (**1u**) was graciously supplied by Ichimoru Pharcos Co. Ltd., Takatomi, Japan. Chimaphilin (**1m**) and 2,3-dimethylnaphthoquinone (**1n**) were prepared by H_2O_2 oxidation of the appropriately substituted naphthalenes by the procedure of Arnold and Larson (15). These naphthalenes were purchased (Carnegie Mellon University and Lancaster Synthesis, Windhorn, NH), and the reaction products were purified by hplc. Phthiocol (**1k**) and 6,7-dimenthoxynaphthoquinone (**1o**) were prepared via Diels-Alder additions (16) from commercially available substrates. Lapachol (**1p**), its isomer (**1q**), and the lapachones (**3** and **4**) were synthesized as described by Hooker (17) and Fieser (18). Again, hplc was used to purify the desired products. Finally, 7-methyljuglone (**1j**) and isodiospyrin (**1w**) were isolated from *Diospyros virginiana* L. wood (19), lomatiol (**1r**) from *Lomatia silaifolia* R. Br. seed (20), cordeauxiaquinone (**1v**) from *Cordeauxia edulis* Hemsl. leaves (21), and cassumunaquinone (**1x**) from *Zinziber cassumunar* Roxb. rhizomes (22). Typically, the extracts were first subjected to silica gel column chromatography to concentrate the compounds of interest, followed by hplc for final purification. Mass and nmr spectra were in agreement with the literature (where available) or were in accord with the proposed structures.

ACKNOWLEDGMENTS

Plant parts were collected or donated by: John Mishell, USAID, Mogadishu, Somalia; Gary Sieren, USDA, National Forest Service, Elizabethtown, Illinois; and Dr. Jack Bond, American Embassy, Thailand. T.S. Wilson assisted with the bioassays.

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Received 15 November 1985