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Antiviral Research 61 (2004) 57–62
Antiviral Research
www.elsevier.com/locate/antiviral

Short communication

Antiviral activity of hop constituents against a series of DNA and RNA viruses

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Received 13 May 2003; accepted 25 June 2003

Abstract

We investigated whether crude hop extracts and purified hop components representing every major chemical class of hop compound have antiviral activity. These hop constituents were tested for antiviral activity against bovine viral diarrhea virus (BVDV) as a surrogate model of hepatitis C virus (HCV), human immunodeficiency virus (HIV), influenza A virus (FLU-A), influenza B virus (FLU-B), rhinovirus (Rhino), respiratory syncytial virus (RSV), yellow fever virus (YFV), cytomegalovirus (CMV), hepatitis B virus (HBV), and herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). The extracts all failed to prevent the replication of HIV, FLU-A, FLU-B, RSV and YFV. A xanthohumol-enriched hop extract displayed a weak to moderate antiviral activity against BVDV (therapeutic index (TI) = 6.0), HSV-2 (TI = >5.3), Rhino (TI = 4.0) and HSV-1 (TI = >1.9) with IC_{50} values in the low μg/ml range. Pure *iso*-α-acids demonstrated low to moderate antiviral activity against both BVDV (TI = 9.1) and CMV (TI = 4.2) with IC_{50} values in the low μg/ml range. No antiviral activity was detected using β-acids or a hop oil extract. Ultra-pure preparations (>99% pure) were used to show that xanthohumol accounted for the antiviral activity observed in the xanthohumol-enriched hop extract against BVDV, HSV-1 and HSV-2. Xanthohumol was found to be a more potent antiviral agent against these viruses than the isomer *iso*-xanthohumol. With Rhino, the opposite trend was observed with *iso*-xanthohumol showing superior antiviral activity to that observed with xanthohumol. Xanthohumol also showed antiviral activity against CMV, suggesting that it might have a generalized anti-herpesvirus antiviral activity. Again, superior antiviral activity was observed with the xanthohumol isomer against CMV. In summary, *iso*-α-acids and xanthohumol were shown to have a low-to-moderate antiviral activity against several viruses. These hop constituents might serve as interesting lead compounds from which more active anti-HCV, anti-Rhino and anti-herpesvirus antiviral agents could be synthesized.

Keywords: Hops; Xanthohumol; *iso*-Xanthohumol; *iso*-α-Acid; Lead compounds; Antiviral

It is widely recognized that the utility of most antiviral agents is limited by the inherent toxicity of the compounds and the appearance of drug resistant virus following their continued use. As such, there is great interest in the identification of novel antiviral agents and lead compounds from which new antivirals can be synthesized. Beer is traditionally prepared from malted barley, hops and water (Broderick, 1977). Hop resins and oils are present in the lupulin glands of the cones of the female hop plant (*Humulus lupulus*) flowers. The hop resins are composed of the α-acids, the β-acids and the remaining "uncharacterized resins" (Palamand and Aldenhoff, 1973; Neve, 1991). Hop oils are a diverse group of over 400 compounds that contribute "hop aroma" to beer. Traditionally whole or compressed hops have been used in

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doi:10.1016/S0166-3542(03)00155-4

the production of beer, however there has been a recent trend towards the use of hop extracts for this purpose. A variety of hop extract products are available including both crude hop extracts and purified hop components containing single chemical species. The use of these extracts may allow for a reduction in costs and for greater consistency in the brewing process.

Many of the major hop components have been characterized (Palamand and Aldenhoff, 1973; Neve, 1991). The principal source of bitter flavor arises from the (*iso*)-α-acids. During the boiling of wort, the virtually insoluble α-acids found in hops are isomerized into the more soluble, bitter and stable *iso*-α-acids. The β-acids are a second class of well-characterized hop components. The hop oils are an extremely diverse group of constituents composed of many unrelated chemical groups (Pfenninger et al., 1979). The rest of the hop resins are a group of generally insoluble and poorly characterized constituents. One component that has been characterized in this group is xanthohumol. This component is converted during wort boiling from the xanthohumol found in hops to *iso*-xanthohumol that is found in beer (Stevens et al., 1999a).

Hop constituents are reported to have a variety of interesting properties including antibacterial activity (Simpson and Smith, 1992), antioxidant activity (Tagashira et al., 1995), and potential anti-cancer activity (Honma et al., 1998). Hops also contain the phytoestrogen 8-prenylnaringenin (Milligan et al., 1999), which has lead to the use of hop extracts as a hormone-replacement therapy in some markets. Furthermore, the hop compound xanthohumol has attracted attention in the brewing community due to reports of potential anti-cancer activity (Stevens et al., 1998; Gerhauser et al., 2002).

The composition of the hop extracts used in this study are shown in Table 1. The CO_2 extract, isomerized kettle extract, xanthohumol-enriched extract, *iso*-α-acids, β-acids and the hop oil preparation were obtained from S.S. Steiner, Inc. (New York, NY). The hop oil used was their steam distilled hop oil which contains all the essential hop oils (~400 compounds). Xanthohumol and *iso*-xanthohumol were purified by HPLC (Stevens et al., 1999a,b).

We tested three crude hop extracts for antiviral activity against a series of diverse RNA and DNA viruses including several important human pathogens (Table 2). The RNA viruses used were BVDV NADL strain in MDBK cells, human influenza A virus (FLU-A, A/Victoria/3/75 (H3N2) strain) in MDCK cells, human immunodeficiency virus (HIV, virus strain HIV-1_{RF}) in CEM-SS cells, human influenza B virus (FLU-B, B/Yamanshi/166/98 strain) in MDCK cells, human rhinovirus (Rhino, rhinovirus 14 strain) in MRC-5 cells, human respiratory syncytial virus (RSV, long strain) in Vero cells, and yellow fever virus (YFV) 17D strain in Vero cells. Antiviral effects of compounds against these RNA viruses were tested using cell-based assays designed to assess inhibition of cytopathic effects (CPE), performed as described previously (Buckwold et al., 2003a) except for the HIV antiviral evaluation assay, which used a different protocol (Weislow et al., 1989). Antiviral positive control compounds were included in every assay. These inhibition of CPE-based assays utilized CellTiter96 reagent (Promega, Madison, WI) to quantitate cell numbers. Ribavirin was the positive control in the BVDV, FLU-A, FLU-B, RSV and YFV assays, while AZT was used in the HIV assay, and Enviroxime (Biota) in the Rhino assay. An in-house computer program was utilized to calculate the mean drug color- and background-corrected percent of CPE reduction of the virus-infected wells and the percent cell viability of uninfected drug control wells in the absence and presence of various dilutions of the drug in question. The minimum inhibitory drug concentration that reduced the CPE by 50% (IC_{50}) and the minimum toxic drug concentration that caused the reduction of viable cells by 50% (TC_{50}) were interpolated from plots generated from the data. A therapeutic index (TI) for each active compound was determined from TC_{50}/IC_{50}. Four human DNA viruses were utilized to assess antiviral effects: cytomegalovirus (CMV, strain AD169 in MRC-5 cells), hepatitis B virus (HBV, using HepG2 2.2.15 cells), herpes simplex virus type 1 (HSV-1, F strain in Vero cells), and HSV type 2 (HSV-2, MS strain in Vero cells). The virus CMV was used in a plaque reduction assay as previously (Krosky et al., 2002). Inhibition of HSV-1 and HSV-2 replication was assessed in inhibition of CPE

Table 1
Composition of hop extracts used for antiviral testing

Hop extract	Extract type	Composition (% by weight)				
		α-Acids	*iso*-α-Acids	β-Acids	Hop oils	Xanthohumol
CO_2 extract	Crude	55	<0.10	19	5–9	<0.10
Isomerized kettle extract	Crude	<2.0	57	20	5–8	<0.10
Xanthohumol enriched	Crude	18	6.0	2.0	<0.10	8.4
iso-α-Acids[a]	Pure	<0.40	30	<0.10	<0.10	<0.10
β-Acids[a]	Pure	<0.10	0.50	9.9	<0.10	<0.10
Hop oil	Pure	<0.10	<0.10	<0.10	>99	<0.10
Xanthohumol	Ultra pure	<0.10	<0.10	<0.10	<0.10	>99
iso-Xanthohumol	Ultra pure	<0.10	<0.10	<0.10	<0.10	>99

[a] Supplied as aqueous potassium salt solutions.

Table 2
Antiviral activities of crude hop extracts against a series of RNA and DNA viruses

Hop extract	Virus	IC_{50}[a,b] (μg/ml)	TC_{50}[a,c] (μg/ml)	TI[a,d] (TC_{50}/IC_{50})
CO_2 extract	BVDV	NR	2.3	<1
	CMV	8.6	13	1.5
	FLU-A	NR	59	<1
	FLU-B	NR	57	<1
	HBV	29	2.9	<1
	HIV	NR	26	<1
	HSV-1	NR	4.1	<1
	HSV-2	NR	4.3	<1
	Rhino	NR	4.2	<1
	RSV	NR	0.40	<1
	YFV	NR	0.32	<1
Isomerized kettle extract	BVDV	NR	5.1	<1
	CMV	4.3	4.3	1
	FLU-A	NR	62	<1
	FLU-B	NR	52	<1
	HBV	22	2.5	<1
	HIV	NR	4.2	<1
	HSV-1	15	3.6	<1
	HSV-2	NR	3.8	<1
	Rhino	NR	3.9	<1
	RSV	NR	1.0	<1
	YFV	NR	1.9	<1
Xanthohumol enriched	BVDV[e]	1.4 ± 0.62	7.8 ± 1.3	6.0 ± 2.0
	CMV	6.9	11	1.6
	FLU-A	NR	40	<1
	FLU-B	NR	65	<1
	HBV	78	40	<1
	HIV	NR	14	<1
	HSV-1[f]	44 ± 33	>50[g]	>1.9 ± 0.99
	HSV-2[f]	11 ± 5.6	>50[g]	>5.3 ± 0.72
	Rhino[f]	6.5 ± 4.1	19 ± 4.0	4.0 ± 2.7
	RSV	NR	3.3	<1
	YFV	NR	5.7	<1

NR: IC_{50} not reached.
[a] Mean ± S.D. values are shown for the active compounds.
[b] IC_{50} is the inhibitory concentration in μM required to reduce viral replication by 50%.
[c] TC_{50} is the toxic concentration in μM required to reduce the number of viable cells by 50%. All compounds were tested at a maximum concentration of 100 μg/ml except as indicated.
[d] Therapeutic index (TI): TC_{50}/IC_{50}.
[e] $n = 4$ independent experiments.
[f] $n = 3$ independent experiments.
[g] Tested at a maximum concentration of 50 μg/ml.

assays as described above (Weislow et al., 1989). Gangciclovir served as a positive control antiviral compound for CMV, while acyclovir was used for both HSV-1 and HSV-2. The HBV HepG2 2.2.15 antiviral assay (Korba and Milman, 1991) used a 3TC positive control.

We first examined the antiviral activity of the crude hop extracts. No antiviral activity was detected against any virus using either the CO_2 hop extract or the isomerized kettle extract (Table 2). The xanthohumol-enriched hop extract displayed weak to moderate antiviral activity against BVDV (TI = 6.0 ± 2.0), HSV-2 (TI = >5.3 ± 0.72), Rhino (TI = 4.0 ± 2.7) and HSV-1 (TI = >1.9 ± 0.99) with IC_{50} values in the low μg/ml range. Since there are no robust methods by which the human hepatitis C virus (HCV) can be grown in vitro, the use of surrogate models of viral replication are often employed. The Pestivirus BVDV is frequently used in the evaluation of the antiviral activity of anti-HCV antiviral compounds (Buckwold et al., 2003a,b). Inhibition of BVDV replication may indicate that the extract has a potential antiviral activity against HCV. The antiviral effects of the crude xanthohumol-enriched hop extract were somewhat specific since the antiviral activity present is not broadly-acting against all RNA or DNA viruses. The other crude hop extracts examined contained little if any xanthohumol. Also the xanthohumol-enriched hop extract does not seem to act non-specifically against all enveloped viruses. BVDV, CMV, HBV, HSV-1, HSV-2, RSV and YFV are the enveloped viruses in this group. Based on the compositional

differences of the various extracts (Table 1) the compound xanthohumol was singled out as the potential candidate constituent responsible for this antiviral activity.

We next examined the antiviral activity of pure hop extracts composed of *iso*-α-acids, β-acids or hop oils against these viruses (Table 3). No antiviral activity was detected using either the β-acids or the hop oil extract. The β-acids were generally cytotoxic at low concentrations. The *iso*-α-acids showed low to moderate antiviral activity against both BVDV (TI = 9.1 ± 8.6) and CMV (TI = 4.2 ± 0.86) with IC_{50} values in the low μg/ml range. There appears to be some specificity to this antiviral activity rather than a broadly-acting antiviral activity since only one enveloped RNA virus (BVDV) and one enveloped DNA virus (CMV) were affected by the compound. It is possible that the crude isomerized hop extract, which is enriched in *iso*-α-acids, did not show antiviral activity against these viruses because its activity was masked by the cytotoxic effects of β-acid fraction that was also present at high concentrations in the extract.

Next we examined the antiviral activity of ultra-pure preparations of xanthohumol and the isomer *iso*-xanthohumol (Fig. 1) against all of the viruses that were inhibited by the xanthohumol-enriched hop extract (Table 4). The xanthohumol alone appeared to account for all of the antiviral activity observed against BVDV, HSV-1 and HSV-2 in the xanthohumol-enriched hop extract since TIs similar to those observed using the crude xanthohumol-enriched extracts were observed. This implicates xanthohumol as the major hop constituent responsible for the antiviral activity of the

Table 3
Antiviral activities of pure hop extracts against a series of RNA and DNA viruses

Hop extract	Virus	IC_{50}[a,b] (μg/ml)	TC_{50}[a,c] (μg/ml)	TI[a,d] (TC_{50}/IC_{50})
iso-α-Acids	BVDV[e]	4.7 ± 5.1	24 ± 12	9.1 ± 8.6
	CMV[f]	9.5 ± 0.50	39 ± 6.1	4.2 ± 0.86
	FLU-A	NR	>100	<1
	FLU-B	NR	99	<1
	HBV	9.2	21	2.3
	HIV	NR	16	<1
	HSV-1	NR	38	<1
	HSV-2	NR	35	<1
	Rhino	NR	21	<1
	RSV	NR	8.1	<1
	YFV	NR	2.0	<1
β-Acids	BVDV	NR	0.81	<1
	CMV	0.13	0.15	1.2
	FLU-A	NR	17	<1
	FLU-B	NR	9.2	<1
	HBV	0.51	0.21	<1
	HIV	NR	1.1	<1
	HSV-1	NR	0.41	<1
	HSV-2	NR	0.40	<1
	Rhino	NR	0.63	<1
	RSV	NR	0.13	<1
	YFV	NR	<0.32	<1
Steam distilled hop oil	BVDV	NR	>100	<1
	CMV	NR	>100	<1
	FLU-A	NR	>100	<1
	FLU-B	NR	>100	<1
	HBV	NR	>100	<1
	HIV	NR	76	<1
	HSV-1	NR	>100	<1
	SV-2	NR	>100	<1
	Rhino	NR	0.36	<1
	RSV	NR	>100	<1
	YFV	NR	>100	<1

NR: IC_{50} not reached.

[a] Mean ± S.D. values are shown for the active compounds.

[b] IC_{50} is the inhibitory concentration in μM required to reduce viral replication by 50%.

[c] TC_{50} is the toxic concentration in μM required to reduce the number of viable cells by 50%. All compounds were tested at a maximum concentration of 100 μg/ml.

[d] Therapeutic index (TI): TC_{50}/IC_{50}.

[e] $n = 7$ independent experiments.

[f] $n = 3$ independent experiments.

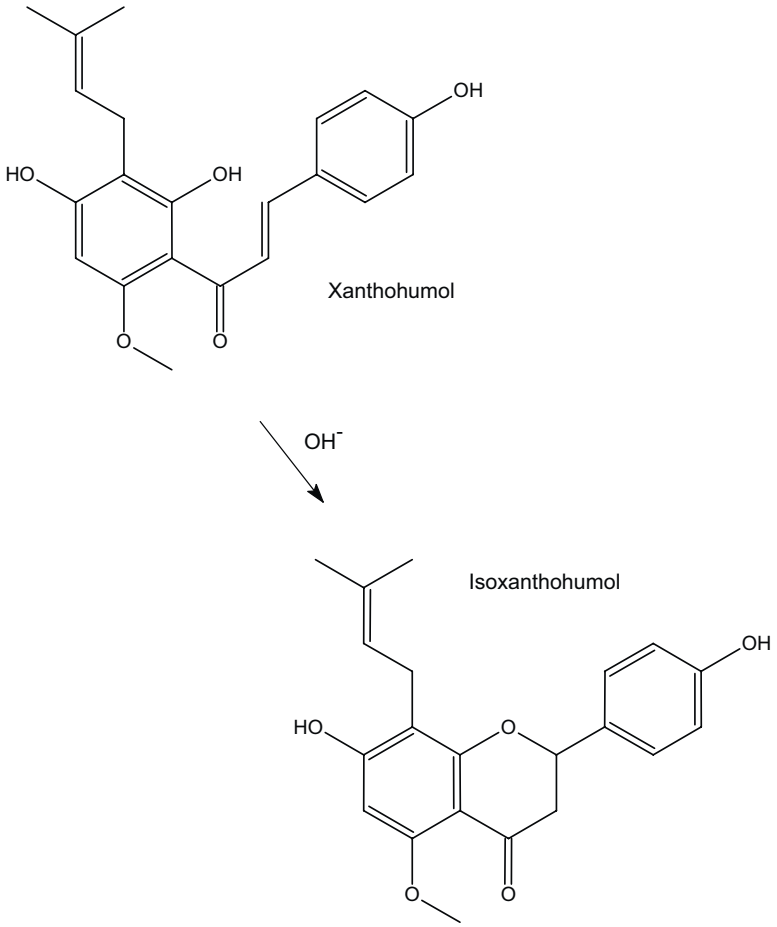

Fig. 1. Structures of xanthohumol and *iso*-xanthohumol. When heated in the presence of base, xanthohumol is converted into *iso*-xanthohumol.

crude xanthohumol-enriched hop extract. However, xanthohumol did not have any antiviral activity against Rhino. This indicates that unidentified compounds apart from xanthohumol were acting against Rhino in the xanthohumol-enriched extract. For BVDV, HSV-1 and HSV-2, the xanthohumol isomer showed superior antiviral activity to that observed with the *iso*-xanthohumol isomer. With Rhino, the reverse trend was true. Since xanthohumol was active against both HSV-1 and HSV-2, we wondered if the pure compound might have a generalized anti-herpesvirus antiviral activity.

Table 4
Antiviral activities of ultra-pure xanthohumol and *iso*-xanthohumol against select viruses

Compound	Virus	IC_{50}[a,b] (μg/ml)	TC_{50}[a,c] (μg/ml)	TI[a,d] (TC_{50}/IC_{50})
Xanthohumol	BVDV	1.7 ± 1.4	6.1 ± 3.3	4.2 ± 0.99
	CMV	2.5 ± 0.56	6.5 ± 3.2	2.6 ± 1.0
	HSV-1	2.7 ± 1.7	7.7 ± 0.57	3.7 ± 1.8
	HSV-2	1.5 ± 0.35	8.9 ± 2.7	6.3 ± 3.3
	Rhino	NR	2.2 ± 0.58	<1
iso-Xanthohumol	BVDV	NR	2.5 ± 1.3	<1
	CMV	12 ± 2.6	25 ± 4.5	2.2 ± 0.81
	HSV-1	NR	62	<1
	HSV-2	17 ± 9.4	55 ± 13	4.2 ± 2.3
	Rhino[e]	6.6 ± 3.3	18 ± 3.3	3.1 ± 1.2

NR: IC_{50} not reached.

[a] Mean ± S.D. values are shown for the active compounds from $n = 3$ independent experiments except as indicated.

[b] IC_{50} is the inhibitory concentration in μM required to reduce viral replication by 50%.

[c] TC_{50} is the toxic concentration in μM required to reduce the number of viable cells by 50%.

[d] Therapeutic index (TI): TC_{50}/IC_{50}.

[e] $n = 4$ independent experiments.

Both isomers showed some antiviral activity against the herpesvirus CMV with a slightly higher activity observed with the xanthohumol isomer.

These results demonstrate that *iso*-α-acids have a moderate antiviral activity against BVDV, a surrogate model of HCV replication, and against CMV. Also, xanthohumol was shown to have a low-to-moderate antiviral activity against BVDV and the herpesviruses (HSV-1, HSV-2 and CMV) that was superior to the antiviral activity of the isomer *iso*-xanthohumol. *iso*-xanthohumol was found to have a weak antiviral activity against Rhino, while xanthohumol had negligible activity against this virus. In summary, *iso*-α-acids and xanthohumol might serve as interesting lead compounds from which more active anti-HCV, anti-Rhino and anti-herpesvirus antiviral agents could be synthesized.

Acknowledgements

This work was supported in part by Southern Research Institute, S.S. Steiner Inc. and an NIH grant AI-53574 to VEB.

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