2,6-Dichlorophenol, Sex Pheromone of the Lone Star Tick

Abstract. *The compound 2,6-dichlorophenol was identified from female lone star ticks,* Amblyomma americanum, *and is believed to be a sex pheromone of this arthropod. The natural occurrence of a chlorinated organic compound in a land animal is new. The presence of similar compounds of exogenous origin is common in animals, but efforts to implicate an outside source for the halogenated phenol have failed.*

Female lone star ticks, *Amblyomma americanum*, contain a chemical that stimulates in males responses which are thought to be related to the mating behavior of the ticks. This substance is apparently a sex pheromone acting to arouse and attract the males (*1*). I report here the identification of this chemical as 2,6-dichlorophenol—an unusual compound to be a natural product in an animal. However, evidence indicates that the compound is produced by the female ticks but does not occur in the hosts' blood or in engorged nymphs. Only trace amounts are found in males.

Each lot of approximately 50,000 adult ticks 2 to 6 weeks old was homogenized in 1 liter of benzene. The homogenate was filtered, and the residue was reextracted twice in a similar manner. The filtrates were combined, dried, and vacuum distilled to remove the benzene. Prior information indicated that the pheromone was a weak acid (*1*). Therefore, the greasy residue (about 1.5 g) was suspended in 0.1*M* NaOH and partitioned with hexane to remove neutral lipids. The resulting aqueous phase was filtered to remove clumped reddish-brown insoluble materials, the filtrate was saturated with CO_2, and the crude pheromone was recovered by extracting it in a small volume of benzene.

Gas chromatography (*2*) of the crude pheromone revealed five major components. Only one of the peaks was active, as determined by observing the reactions of male ticks attached to a host to the column effluent (*1*). Approximately 250 μg of pheromone was obtained from a sample of 50,000 ticks. The pheromone needed for determination of the infrared and mass spectra was obtained by making larger injections and collecting the material in the active peak.

The infrared spectrum of the pheromone in CCl_4 and CS_2 has peaks at 2.8 μm (free OH); 7.55 and 8.06 μm (C–O–H); and 6.33, 6.85, and 6.91 μm (aromatic), which indicates that the compound is a phenol. Several peaks in the region from 8 to 14 μm are consistent with a molecule having three adjacent ring hydrogens. A sharp peak at 12.6 μm is probably the result of C–Cl stretching. The largest peak in the mass spectrum is due to the parent ion (P), which has a mass-to-charge ratio, *m/e*, of 162. Two large peaks at P + 2 (164) and P + 4 (166) are 66 and 12 percent as large as the peak for the parent ion, respectively. These are due to ^{37}Cl and suggest two atoms of Cl. The mass spectra of dichlorophenols are not sufficiently different that the individual isomers can be readily identified. The pheromone was identified specifically as the 2,6-isomer by comparing its infrared spectrum and gas chromatographic properties with those of known samples of all the possible isomers.

Synthetic 2,6-dichlorophenol elicited behavioral responses in males similar to those observed with female tick extract. Although no efforts were made to determine the minimum quantity of phenol that the ticks can detect, good responses were observed when 0.1 μg of the compound was injected into the gas chromatograph column and approximately half of the effluent gases was directed over the ticks. Limited tests in which 10 μg of the compound was applied on a host indicated that the phenol promoted attachment of males on the host, but did not appear to affect the attachment of females. Males attached at one position on a host were induced to move to a new location after it was treated with 10 μg of the compound, especially when other males were already attached at the site of application of the chemical. Feeding females did not exhibit this response.

Although the compound exhibits many of the physiological properties normally connected with sex pheromones, its structure is not easily accepted as being that of a natural product of an arthropod. Phenol has been identified as a sex pheromone of the grass grub beetle, *Costelytra zealandica* (*3*), but the natural occurrence of chlorinated organic compounds in land animals is unknown. Only certain fungi are known to produce them (*4*). However, chlorinated industrial and agricultural chemicals are widely distributed in the environment and are sometimes found in unexpected places. For example, 2,5-dichlorophenol has been identified in the defensive secretions of grasshoppers and is thought to stem from 2,4-D (2,4-dichlorophenoxyacetic acid) or a metabolite of it that was ingested by the insects with their food (*5*). Also, 2,6-dichlorophenol or an appropriate precursor might have been passed along the food chain of the ticks. If this were true, we might expect to find detectable amounts of the compound in the blood of host animals and in engorged nymphs prior to the emergence of the adults. But this is not the case. None of the compound was detected in the blood of host rabbits or in engorged nymphs by using gas chromatography with electron capture detection, which is very sensitive for 2,6-dichlorophenol. The compound begins to appear in female ticks 4 to 5 days after emergence and seems to reach a maximum of about 7.0 parts per million (ppm) in about 2 weeks. Quantities near the lower limits of detection (0.1 ppm) of the analytical procedure have been found in some samples of males.

It is possible that the 2,6-dichlorophenol is ingested as a part of a larger molecule that is degraded by the females. Therefore, the nymphal extracts were analyzed at a higher column temperature to determine if a strong electron-capturing compound, which might have a retention time longer than that of 2,6-dichlorophenol, was present. No peak was found to support this idea. Hydrolysis of the extracts with alkali failed to liberate detectable amounts of the phenol. It is possible that the extracting solvent (benzene) would not dissolve certain precursors, especially highly polar compounds. Homogenates of nymphs were hydrolyzed also, but these showed no evidence of the phenol either.

In conclusion, it does not appear that the ticks acquire the chlorinated phenol from an external source. Apparently it is a sex pheromone for this arthropod and is synthesized by the female.

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References and Notes

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2. A column (4 mm by 1.9 m) of 10 percent Carbowax 20M on Anakrom ABS (60/70 mesh) was held at 175°C; the carrier gas was N_2 (60 cm^3/min). The retention time of the pheromone was 6.8 minutes.
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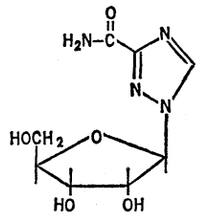
6. Supported in part by PHS grant AI-07742 from the Institute of Allergy and Infectious Diseases.

30 March 1972; revised 5 June 1972

Broad-Spectrum Antiviral Activity of Virazole: 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide

Abstract. *Virazole is a synthetic nucleoside active in tissue culture against at least 16 DNA and RNA viruses. Applied topically, it inhibits herpetic keratitis in rabbits and tail lesions induced by herpes, vaccinia, and vesicular stomatitis viruses in mice. Injected intraperitoneally into mice, it inhibits splenomegaly and hepatomegaly induced by Friend leukemia virus and respiratory infections caused by influenza A_0, A_2, and B viruses and parainfluenza 1 virus. Oral or aerosol treatment of parainfluenza virus infections is also effective.*

Few chemicals have been reported which have broad-spectrum antiviral activity, and the majority of these are inducers of interferon, hence effective primarily as prophylactic rather than therapeutic agents. No synthetic compounds are known which have significant in vitro and in vivo antiviral activity against both DNA and RNA viruses. We describe the broad-spectrum antiviral activity of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole, ICN-1229), a water-soluble, stable, colorless nucleoside, the synthesis of which has been reported (*1*).



Virazole

In tissue culture experiments, inhibition of virus-induced cytopathogenic effect was determined in the appropriate cell lines grown in disposable plastic microplates as described (*2*). Inhibition of cytopathogenic effect was evaluated by the virus rating method (*3*). Inhibition of virus production, as measured by extra- and intracellular virus titers (*2*), was also studied. Because the influenza viruses produced little discernible cytopathogenic effect in the cells used, reduction in supernatant hemagglutination titers, expressed as therapeutic index (*4*), was used to determine antiviral effect. As judged by these measurements, Virazole was markedly inhibitory to both RNA and DNA viruses (Table 1). Drugs known to be active against one or more of the viruses listed in Table 1 were tested against those viruses in experiments run parallel to those with Virazole. In most of these comparative evaluations, Virazole had antiviral activity equal to, or greater than, the known active compound (*5*), and its spectrum of activity exceeded that of all of the compounds studied.

To determine if Virazole induced interferon, we evaluated the chemical both in vitro (*2*) and in vivo. Virazole-free medium collected 24 or 48 hours after a 3-hour incubation of L-929 cells with the compound (1000 μg/ml) contained no interferon-like activity against vesicular stomatitis virus. The cells in this experiment were not rendered refractory to infection. In the in vivo system, male Swiss-Webster mice (18 to 22 g) were injected once intraperitoneally with Virazole (1000 mg per kilogram of body weight), and serums collected from these animals 2 to 24 hours later were assayed for interferon activity in L-929 cells. No substance inhibitory to vesicular stomatitis virus was found in these serums, a result indicating that Virazole was not an inducer of interferon. The compound, tested as described (*2*), was not virucidal against herpes simplex or parainfluenza virus.

The following animal experiments were designed to determine if the broad in vitro antiviral activity would also be seen in vivo. The corneal epithelia of both eyes of New Zealand albino rabbits (3 to 5 kg) were uniformly scratched, and two drops of a suspension containing herpes simplex virus (McKrae) of known titer were instilled in each. One eye of each rabbit was treated with Virazole for 7 days. Virazole (10, 1, or 0.1 percent), dissolved in 1.4 percent polyvinyl alcohol, was applied hourly from 8 a.m. to 7 p.m., and at 8 p.m. eyes were treated with Virazole suspended in Jellene base ophthalmic ointment containing 1 percent chloramphenicol (Parke-Davis, Detroit). Treatment began 4 hours after virus inoculation. The remaining eye of each rabbit was similarly treated with polyvinyl alcohol and ointment devoid of Virazole to serve as control. Each eye was examined daily, both grossly and by biomicroscope, on a blind basis for infectivity (lesion size and type, corneal opacity) and for inflammatory response (erythema, chemosis, discharge), and the weighted grading scale described by Corwin *et al.* (*6*) was used. The eyes treated with 10 or 1 percent Virazole showed significant improvement ($P < .01$, as determined by ranking analysis) over placebo-treated eyes. This improvement was evident from both the weighted scale and from each individual scoring method. Uninfected, treated controls examined in parallel with infected animals revealed no toxic effects in any of the treated eyes.

Virazole was evaluated in the system of virus-induced mouse tail lesions described by Yoshimura *et al.* (*7*). A 20 percent solution of the compound in polyvinyl alcohol, applied topically to the tail twice daily for 15 days (the first application 18 hours after virus inoculation), inhibited the development of the lesions induced by herpes simplex, vaccinia, and vesicular stomatitis viruses. The probability values (*8*) for this lesion inhibition were $< .01$ for the period between 5 and 30 days after virus inoculation. No skin irritation was seen in uninfected, treated animals.

Splenomegaly and hepatomegaly induced by Friend leukemia virus (*9*) was significantly inhibited by intraperitoneal treatment with Virazole (100 mg/kg) administered twice daily for 9 days, with the first injection 4 hours before virus inoculation. Total body weights, as well as spleen and liver weights, were determined on day 14;