cyclooctene[1], cyclononene[2], and cyclodecene[3]. From the published physical constants the rule predicts that with the eight-membered ring the *cis* isomer would be the more stable, while the reverse would be true in the ten-membered ring and the isomers in the nine-membered ring would have nearly equal stabilities. The same orders of stability were found by chemical methods.

It is to be noted, however, that applications of the rule to olefins can be made with certainty only with mono-olefinic hydrocarbons. The reason for this limitation is that a double bond possesses electronic properties not shared by the other ring compounds and, in the presence of certain functional groups, interactions can occur which will overshadow the differences in the dispersion forces. As an example, 2-alkenoic acids are, as a class, known to be exactly reversed from the hydrocarbons with respect to the properties previously mentioned.

N. L. ALLINGER

Department of Chemistry, University of California, Los Angeles 24, California, February 26, 1954.

Zusammenfassung

Eine Neufassung der von-Auwerschen Regel ist vorgeschlagen worden. Die neue Regel ist auf *cis-* und *trans-*Isomeren gewöhnlicher zyklischer Systeme sowie auch auf mehrfach substituierte zyklische Verbindungen, polyzyklische Verbindungen und Olefine anwendbar. Das Bestehen einer solchen Regel ist begründet, und ihre Begrenzungen und praktischen Auswertungen sind unter Betracht gezogen worden.

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On the Chemical Nature of Acerin and the Virucidal and Antiviral Effects of Some Vegetable Tannins[1]

In a search for virucidal and antiviral substances in nature, one of us (G.F.) in 1952[2] found that water extracts of several plants exerted a definite virucidal effect, the main experiments being performed with a bacterial virus and its host cell, *Escherichia coli* XP. Out of 300 different plant species investigated, the majority yielded no activity, whereas fairly active extracts were obtained from some of them.

In his next communication, FISCHER[3] reported on the virucidal and antiviral effect of maple fruit preparations. 25 g of maple fruits gave 1 g of a substance, which in a dilution of 1:64 destroyed the bacterial virus in 5 min. The substance was given the name *acerin*.

Acerin also destroyed vaccinia virus in 5 min; its effect on the Staphylococcus Twort virus was much less pronounced.

After further purification (FISCHER[1]) whereby 100 g of maple fruits yielded 0·75 g, the purified product destroyed the bacterial virus within 5 min in a dilution of 1:50,000, a concentration which allowed an abundant development of the coli organisms. The lowest concentration of acerin which exerted a slight colistatic effect was 1:200. Acerin was active only on the extracellular virus. A good antiviral effect on the virus host cell system was obtained in broth culture and even on solid media.

It was found that the active principle precipitated when increasing amounts of sodium chloride were added to the solution. Its virucidal effect was neutralized when it was exposed to serum diluted 1:2–32. In a concentration of 1:800 it also gave a precipitate with normal serum.

In discussing the possible chemical nature of the active principle in the acerin preparations, we were struck by the similarity in the properties of acerin to those of the vegetable tannins. The general occurrence of tannins in fruits and plant juices also contributed to our supposition that the active principle might be a tannin. It seemed tempting to ascribe the virucidal action of the acerin preparations to a tanning effect on the virus protein. The peculiar denaturation of the protein molecule caused by the tanning substances would provide a satisfactory explanation of the inactivation of the virus. Furthermore, the non-dialyzable tannins cannot reach the inside of the cell wall on the bacteria and would therefore act only on extracellular virus particles, as was the case with the acerin preparations. Moreover, the active principle could be precipitated with sodium chloride as can the tannins. Its neutralization in the presence of serum is also to be expected when serum proteins react with the tanning principle. As found by FISCHER, the strongest acerin preparation had a reddish-brown colour, in similarity to tannins, after treatment with alkali and subsequent oxidation in air. They also gave a greenish-brown precipitate with ferric chloride.

The virucidal effect of some vegetable tannins. It was found that several of the vegetable tannins exerted a virucidal action similar to that of the strongest acerin preparations. Eighteen natural tannins were analyzed[2]. In about the same concentration as the purest acerin preparations, 1:25,000, six of them, i.e., commercial tannic acid, Mimosa, ground Canaigre root, powdered Canaigre extract, Quebracho and Babul bark, killed the bacterial virus within 5 min.

Experimental. Both the pure tannic acid and the crude tannin preparations were dissolved in water. Any insoluble residue was removed and the solution dialyzed for 48 h at room temperature against distilled water, the outer liquid being exchanged several times. The content of the bags was then freeze-dried. The dry powder was analyzed for virucidal and bactericidal action as described earlier[3].

[1] While this paper in print, our attention was called to a recent publication in J. Bact. (*66*, 572 1953) by CARSON and FRISCH about the growth inhibiting action of tannic acid on influenza virus *in ovo*. We were ignorant of this work and of the previous publication from 1948 by GREEN, Proc. Soc. Exptl. Biol. Med. *67*, 483 (1948).

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[1] G. FISCHER, *Further investigations on the virucidal and anti-virotic effect of a maple-fruit preparation-Acerin (Ac_{50}), performed with a coli culture and its bacterial virus*, Acta Pathol. Microbiol. Scand. No. *1381* (1954) (in Korrektur).

[2] We wish to express our thanks to Mr. K. H. GUSTAVSON, Doctor of Technology, of The Research Institute of the Swedish Tanning Industry, Stockholm, for his assistance and valuabel advice, as well as for his kindness in placing all the tannin preparations at our disposal.

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Substance	Bacterial virus (Exposed for 5 min)		*E. coli* (Exposed for 5 min)		*E. coli* (Exposed for 3 h)		50% serum	
	Virucidal in conc.	Non-virucidal in conc.	Bactericidal in conc.	Non-bactericidal in conc.	Bactericidal in conc.	Non-bactericidal in conc.	Precip. by conc.	Not precip. by conc.
Acerin	1:25,600	1:51,200		1:100		1:100	1:200	1:400
Mimosa	1:25,600	1:51,200		1:100	1:100	1:200	1:1,600	1:3,200
Myrobalans	1:3,200	1:6,400		1:100		1:100	1:200	1:400
Spruce bark extract (Picea excelsa)	1:6,400	1:12,800		1:100		1:100		1:100
Sumac (Rhus coriaria)	1:6,400	1:12,800		1:100		1:100	1:800	1:1,600
Oak wood extract (Quercus robur)	1:3,200	1:6,400		1:100		1:100	1:100	1:200
Gambier	1:800	1:1,600		1:100		1:100		1:100
Chestnut wood extract (Aesculus)	1:6,400	1:12,800		1:100		1:100	1:100	1:200
Valonia extract	1:1,600	1:3,200		1:100		1:100		1:100
Ground Canaigre root	1:51,200	1:102,400		1:100		1:100	1:800	1:1,600
Quebracho	1:25,600	1:51,200		1:100		1:100	1:800	1:1,600
Sulphited quebracho	1:12,800	1:25,600		1:100		1:100	1:200	1:400
Konnan bark extract (*Cassia Pistula*)	1:1,600	1:3,200		1:100	1:100	1:200	1:400	1:800
Divi Divi extract (*Caesalpina coriaria*)	1:400	1:800		1:100		1:100	1:800	1:1,600
Avaram bark extract (*Cassia Auriculata*)	1:1,600	1:3,200		1:100	1:400	1:800	1:800	1:1,600
Babul bark extract	1:51,200	1:102,400		1:100	1:200	1:400	1:400	1:800
Powdered Canaigre extract	1:25,600	1:51,200		1:100		1:100	1:800	1:1,600
Tannic acid	1:25,600	1:51,200		1:100		1:100	1:800	1:1,600
"Desivon" (Astra)	1:800	1:1,600	1:12,800	1:25,600			1:3,200	1:6,400
$HgCl_2$	1:800	1:1,600	1:12,800	1:25,600			1:400	1:800

Results. As shown in the Table, all the tannins in vestigated had a considerable effect on the bacterial virus. Death occurred after exposure for 5 min to tannins in concentrations between 1:50,000 and 1:400.

On the other hand, none of the tannins had a bactericidal effect on the host cell after exposure for 5 min. With an exposure time of 3 h the great majority of the tannins had no bactericidal effect in a dilution of 1:100, which was the lowest concentration tested.

The tannins thus combined a strong lethal effect on the bacterial virus with a slight bactericidal action, or none at all, on the host cell. In similarity to acerin, a particularly powerful virucidal effect was exhibited by some of the tannins, i.e., Mimosa, ground Canaigre root, powdered Canaigre extract, Quebracho, Babul bark extract and tannic acid.

Discussion. In reviewing the literature on the action of disinfectants and other substances on phage, one of us (G.F.) pointed out that D'HERELLE had long since found quinine-containing culture media to have a definite phagicidal effect, ridding the host strain growing on them of its phage. The same goal can apparently be reached with the use of certain vegetable tannins in low concentrations instead of quinine. Consequently, further detailed tests are indicated to ascertain whether or not vegetable tannins can be used to destroy phagal contaminations in bacterial cultures.

It is also interesting to speculate on the extent to which the vegetable tannins are able to exert a protective action against viral infections in the plants, similar to the virucidal effect on the *E. coli* phage demonstrated in the present experiments.

G. FISCHER, S. GARDELL, and E. JORPES

Department of Hygiene and the Chemistry Department, Karolinska Institutet, Stockholm, February 23, 1954.

Zusammenfassung

Die viruzide Wirkung von Extrakten aus Ahornsamen auf Coli-Bakteriophagen ist durch Tannine bedingt. Verschiedene Gerbstoffe zeigen einen zum Teil sehr starken virushemmenden Effekt, wobei nur das extrazelluläre Virus angegriffen wird. Wahrscheinlich handelt es sich um eine Gerbwirkung auf das Protein des Virus.

Die pflanzlichen Gerbstoffe tragen vermutlich dazu bei, die Pflanzensäfte virusfrei zu halten.

Ergebnisse der Flimmer-Elektroretinographie[1]

Das Bestreben, zu Untersuchungsmethoden zu gelangen, die unabhängig von den Angaben oder dem Verhalten des Versuchsobjektes den Reizerfolg aufzuzeichnen gestatten, hat bei der Netzhaut dazu geführt, die auftretenden Aktionspotentiale zu registrieren. Von den hierzu entwickelten Verfahren erlaubt die bei plötzlicher Belichtung bzw. Verdunklung ableitbare Veränderung des korneo-retinalen Ruhepotentials einen Einblick in die retinale Durchschnittsaktivität, während bei Ableitung von Fasern des *Nervus opticus*[2] oder von Retinaganglienzellen[3] das Verhalten einzelner Netzhautelemente studiert werden kann. Da beim Menschen nur die erstgenannte Methode anwendbar ist, konnten hier bisher nur solche Funktionen geprüft werden, die die Erregungen grösserer Teile der Netzhaut wiedergeben. Genauere Untersuchungen des Gesichtsfeldes oder der Sehschärfe gestalten sich daher mit dieser Methode recht schwierig, während sich zum Beispiel der Verlauf der Dunkeladaption sehr wohl darstellen lässt[4]. Trotz dieser Einschränkungen erscheint die Methode des Elektroretinogramms (ERG.) durchaus noch erweiterungsfähig. So lässt sich bei Verwendung intermittierender Lichtreize zeigen, dass die Stäbchen- und Zapfenaktivität der Säugetiernetzhaut sowohl qualitativ (an der Form der auftretenden Aktionspotentiale) als auch quantitativ (durch Bestimmung der Verschmelzungsfrequenz) unterschieden werden kann[5].

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