Table IV. Balance studies with apples treated with C^{14}-GS 13005

Fractions	% of recovered radioactivity								
	Days after treatment								
	0	2	6	10	15	21	30	42	56
CO_2	0	1.1	5.1	11.2	17.9	23.6	26.6	30.5	31.6
Acetone wash	98.7	59.8	38.8	30.0	18.3	5.0	4.6	1.1	2.2
Acetone extract	0.6	33.1	52.2	48.7	34.6	48.4	38.8	40.7	35.6
Residue	0.2	2.1	4.6	7.4	15.1	11.9	11.4	20.2	12.7
Total	99.5	96.1	100.7	97.3	85.9	88.9	81.4	92.5	82.1

Material as in Table I. Dose: 0.369 μc per apple, topically applied in 0.1 ml of acetone. Storage of the apples in a dessicator connected to an air pump; the apple was rinsed with acetone prior to homogenization in acetone; the activity not extractable from the residue was determined by combustion.

ten days that the acetone extract of the total fruit contains some unchanged material.

Résumé. Le métabolisme de l'insecticide GS 13005 (marqué par le C^{14} dans l'hétérocycle) fut étudié chez l'animal et dans la plante. Une conversion considérable de cette molécule en CO_2 fut constatée dans tous les deux. En plus aucune accumulation de l'insecticide intact ou de ses produits métaboliques n'a pu être observée, ni dans la plante, ni dans l'animal.

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Some Relations Between the Structure and the Antibacterial Activity of Natural Coumarins

It is a well-known fact that dicoumarol and anticoagulants in general retard the growth of Gram-positive microorganisms. Little, however, is known about the antibacterial effects of natural coumarins[1,2]. As was ascertained in our previous experiments[3], out of eighteen natural coumarins and furanocoumarins, only ostruthin (6-geranyl-7-hydroxycoumarin) shows a strong effect specifically on Gram-positive organisms. The present report gives the results of our investigation on the effect of further related coumarins and furanocoumarins on Gram-positive and Gram-negative organisms. We have tried to ascertain the influence of the side isoprenic chain and of phenolic hydroxyl on the antibacterial activity.

Coumarins used[4]: (1) coumarin, (2) umbelliferone (7-hydroxycoumarin), (3) osthol (7-methoxy-8-isopentenylcoumarin), (4) ostruthin (6-geranyl-7-hydroxycoumarin), (5) ostruthin-methylether, (6) umbelliprenin (7-farnesoxycoumarin), (7) ammoresinol (3-farnesyl-4:7-dihydroxycoumarin), (8) diacetylammoresinol, (9) ammoresinoldimethylether, (10) dicoumarol (3:3'-methylene-bis-(4-hydroxycoumarin).

Furanocoumarins used[4]: peucedanin (11-methoxy-10-isopropylpsoralene), imperatorin (8-isopentenyloxypsoralene), isoimperatorin (5-isopentenyloxypsoralene), oxypeucedanin (5-(2':3'-epoxy)-isopentyloxypsoralene), ostruthol (5-(3'-hydroxy-2'-angelicyloxy)-isopentyloxypsoralene).

Alcoholic solutions of coumarins and furanocoumarins were tested on agar plates in a concentration of $5 \cdot 10^{-3} M$ by means of the paper disc method[3].

Microorganisms used: of the Gram-positive microorganisms, members of the genera Staphylococcus, Micrococcus, and Bacillus. Gram-negative organisms were represented by *Escherichia coli*, *Aerobacter aerogenes*, and *Serratia marcescens*.

The results are given in the Table. With the exception of the slight effect of peucedanin on Gram-positive organisms, all furanocoumarins were ineffective and are therefore not mentioned in the Table. Similarly, we do not mention in the Table Gram-negative organisms which are not affected by any of the preparations used.

It follows from the results obtained that all effective derivatives, similarly dicoumarol, act selectively on Gram-positive microorganisms. The greatest effect was shown by ostruthin (4) and ammoresinol (7). In order to produce the effect of ostruthin, the presence of the side chain is necessary. Umbelliferone (2) has no effect at all. Methylation of the –OH group in ostruthin results in a weakening of the inhibitory effect (5), but not in its nullification. The presence of phenolic hydroxyl is not the

[1] P. K. BOSE, J. Indian chem. Soc. *35*, 367 (1958).
[2] D. P. CHAKRABORTY, M. SEN, and P. K. BOSE, Trans. Bose Res. Inst. *24*, 31 (1961).
[3] V. DADÁK, Československ. farm. *7*, 394 (1958).
[4] The derivatives characteristic of the family Daucaceae were obtained thanks to Prof. Dr. F. WESSELY, Universität Wien, peucedanin thanks to Prof. Dr. H. SCHMID, Universität Zürich, the methylated and acetylated derivatives were prepared according to SPÄTH[5].
[5] E. SPÄTH, A. J. SIMON, and J. LINTNER, Ber. dtsch. chem. Ges. *69*, 1656 (1936).

main cause of the effect of ostruthin. An antibacterial effect is also shown by osthol (3) containing methoxyl and an unsaturated side chain consisting of five C atoms. The mere presence of the isoprenic side chain consisting of fifteen C atoms and being etherically bound on a coumarin molecule (umbelliprenin 6) fails to show any inhibitory effect.

In contrast to ostruthin, the retaining of free –OH groups is necessary for the effect of ammoresinol (7). Their acetylation results in a marked decrease of its effect (8), their methylation resulting in a complete nullification of the effect (9). An important role is probably that played by hydroxyl on the fourth carbon, as can be seen from the effect of dicoumarol (10).

The effect of natural coumarins on Gram-positive microorganisms

Micro-organism	Derivative (conc. $5 \cdot 10^{-3} M$)									
	1	2	3	4	5	6	7	8	9	10
S. aureus										
NCTC 8532	0	0	(+)	++	(+)	0	++	(+)	0	+
BS 270	0	0	(+)	++	0	0	++	(+)	0	+
M. luteus										
ATCC 398	0	0	(+)	++	+	0	+	(+)	0	+
BS 852	0	0	+	++	+	0	++	(+)	0	+
BS 1682	0	0	(+)	++	+	0	++	+	0	+
M. lysodeicticus										
ATCC 4698	0	0	(+)	++	+	0	+	(+)	(+)	+
ATCC 12698	0	0	(+)	++	+	0	+	+	0	++
NCTC 2665	0	0	(+)	++	++	0	+	(+)	0	+
NCTC 7011	0	0	(+)	++	+	0	++	(+)	0	++
Bac. megatherium										
M	0	0	+	++	++	0	++	(+)	0	++

++ = inhibition zones clear, with clear-cut demarcation; + = inhibition zones with indistinct demarcation; (+) = inhibition zones dull, partly outgrown.

The quantitative evaluation of the effect expressed in terms of the lowest effective concentration in the plate test has shown that ammoresinol is the most effective derivative. In *S. aureus* NCTC 8532 it causes marked inhibition in a concentration of $1 \cdot 10^{-4} M$ and is 2.5 times as effective as ostruthin and dicoumarol. On *M. luteus* BS 852, *M. lysodeicticus* ATCC 12698, NCTC 7011 and *S. aureus* BS 270, ammoresinol is effective up to $5 \cdot 10^{-5} M$ and is five times as effective in these cases as ostruthin and dicoumarol. *Bac. megatherium* is affected by ammoresinol up to $2.5 \cdot 10^{-5} M$ and the latter is four times as effective as ostruthin and dicoumarol.

The testing of ostruthin, ammoresinol and dicoumarol in a mixture with a double surplus of vitamin K_1 (phylloquinone), K_2 (farnoquinone), and K_3 (menadiol solubile) has shown that it is only vitamin K_2 that partly reduces the effect of ostruthin in all the strains tested with the exception of *Bac. megatherium*. The antagonistic effect of vitamin K_2 on ammoresinol was even weaker than that on ostruthin. In the case of dicoumarol, its inhibitory effect in a mixture with the above vitamins remained the same.

The selective effect of natural coumarins indicates an effect of the latter substances on metabolic processes in Gram-positive bacteria. It is possible that vitamin K_2 may be involved in these processes[6].

Zusammenfassung. Von 15 untersuchten natürlichen Cumarinen und Furocumarinen besitzen Ammoresinol und Ostruthin stärkste Wirkung auf Gram-positive Mikroorganismen. Zur Ammoresinolwirkung ist die Erhaltung der freien –OH-Gruppen notwendig, während bei Ostruthin durch Vitamin K_2 die Wirksamkeit vermindert wird.

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January 7, 1965.

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[7] The authors wish to thank Mrs. M. Kazdová for her technical assistance.

Über die Abhängigkeit der Acetylcholinwirkung von der äusseren Ca-Konzentration bei isolierten Meerschweinchenvorhöfen

Die wesentliche Wirkung von Acetylcholin (ACh) am Herzen wird heute in einer spezifischen Erhöhung der Membranpermeabilität für K-Ionen gesehen[1]. Die Folge davon ist eine Verkürzung der Aktionspotentialdauer, die ihrerseits für die negativ inotrope Wirkung von ACh verantwortlich gemacht wird. Hoditz und Lüllmann[2] und Grossman und Furchgott[3] konnten zeigen, dass der ^{45}Ca-Umsatz von elektrisch gereizten Meerschweinchenvorhöfen durch ACh vermindert wird. Diese Autoren nehmen daher an, dass infolge der verkürzten Aktionspotentialdauer die für die Auslösung der Kontraktion notwendige Ca-Ionenkonzentration im Zellinneren während der Erregung nicht erreicht wird. Durch die vorliegenden Versuche soll gezeigt werden, dass Veränderung der extrazellulären Ca-Konzentration ($[Ca]_e$) die negativ inotrope Wirkung von ACh beeinflusst.

Die Versuche wurden an isolierten, elektrisch gereizten (160/min) linken Meerschweinchenvorhöfen durchgeführt. In einer Versuchsserie wurden die Kontraktionen der Vorhofpräparate auf einem Russkymographion isotonisch registriert. Hierbei wurde ACh der Badlösung zugesetzt, so dass Endkonzentrationen von 10^{-8}, 10^{-7} und 10^{-6} g/ml resultierten. Die Einwirkungsdauer der Substanz betrug 10 min. In einer zweiten Versuchsserie wurde die Kontraktionskraft der Vorhöfe über eine Transducerröhre (RCA 5734) isometrisch registriert, gleichzeitig wurden Ruhe- und Aktionspotentiale mit Glasmikroelektroden intrazellulär abgeleitet. ACh wurde 10 min lang mit konstanter Geschwindigkeit in das Organbad infundiert. Als

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[3] A. Grossman und R. F. Furchgott, J. Pharmacol. exp. Therap. *145*, 162 (1964).