INSECTICIDAL PROPERTIES AND CHEMICAL CONSTITUTION

Part III. Some Partial Methyl Ethers of Hydroxyflavones

By T. R. Seshadri and N. Viswanadham
(From the Department of Chemistry, Andhra University, Waltair)

Received February 5, 1947

A number of partial methyl ethers of flavones and flavonols occur in plant drugs. In several cases they seem to be the only special components present. Examples are calycopterin from the leaves of *Calycopteris floribunda*, gardenin from the gum of *Gardenia lucida*, tambulin from the seeds of *Zanthoxylum acanthopodium* and oroxylin-A from the root bark of *Oroxylum indicum*. Very little is known about the physiological properties of these compounds. Among these plant drugs the copper coloured terder leaves of Calycopteris floribunda have been reputed to have anthelmintic properties.[1] In their account of the isolation of calycopterin from this source, Ratnagiriswaran, Sehra and Venkataraman[2] stated that this compound had been found to be toxic to round worms and clinical trials conducted in the General Hospital, Madras, had given encouraging results. Later Mahal[3] could not confirm these findings using as experimental animals round worms, tape worms and leeches. In view of the interesting results reported in Part I[4] of this series of papers that ethers of simple hydroxy flavones exhibit definite toxicity to fish, the toxic properties of calycopterin and its methyl ethers have now been investigated using these animals.

From the experimental data given later it will be clear that calycopterin and its mono and dimethyl ethers are definitely toxic to fish. Calycopterin dimethyl ether is markedly more toxic than the isomeric hexamethyl ethers of myricetin and quercetagetin. It has already been pointed out that the presence of more than one methoxyl in the side phenyl nucleus diminishes toxicity markedly. But calycopterin dimethyl ether (calycopteretin-hexamethyl ether) is even more toxic than the pentamethyl ether of herbacetin which like it has also only one methoxyl group in the side-phenyl nucleus. Hence it appears that the presence of one more methoxyl in the benzo-pyrone part enhances toxic properties. Calycopterin itself is less toxic than its dimethyl ether as could be normally expected.

The most interesting data are provided by the two isomeric mono-methyl ethers of calycopterin. The 4′-monomethyl ether having a free

hydroxyl in the 5-position is markedly more toxic than even the dimethyl ether and the 5-mono-methyl ether on the other hand is only feebly toxic. Thus a free hydroxyl in the 5-position seems to be desirable for enhanced toxicity and a free hydroxyl in the side-phenyl nucleus (4′-position) appears to be unfavourable. These points seem to be supported by some of our past experiments. For example, galangin (3:5:7-trihydroxy flavone) was found to be much more toxic than 3:7-dihydroxy flavone and kæmpferol (3:5:7:4′-tetrahydroxy flavone) had only feeble toxicity. Again chrysin, as shown in this paper, is considerably more toxic than 7-hydroxy flavone. The moderate toxicity of calycopterin is obviously the resultant effect of the two hydroxyls in the 5 and 4′-positions superimposed on the completely oxidised benzopyrone structure and this may be an advantage in an anthelmintic which has to be administered internally. Further this structure appears to confer on the compound a desirable degree of water and lipoid solubility.

Among the number of partial methyl ethers that were examined in the course of this work there are some simpler compounds that have a free hydroxyl group in the 5-position. They do not exhibit the toxic properties so markedly shown by calycopterin and its 4′-methyl ether. It should therefore be concluded that the correlation between structure and toxic properties is complex and cannot be analysed in a simple manner. A comparison of 7-hydroxy flavone with 3:7-dihydroxy flavone and 3-methoxy-7-hydroxy-flavone and of chrysin with galangin and its 3-methyl ether would indicate that the addition of a hydroxyl in the 3-position of these simple structures is favourable to toxicity and that its methylation is a disadvantage.

In our attempts to prepare calycopterin we had occasion to examine the mature green leaves of *C. floribunda* obtained from the Cuddappah district of the Deccan plateau. The ether and acetone extracts of these leaves were definitely toxic to fish and they were found to contain calycopterin. Extraction using the method of Shah *et al.*[7] revealed that these form a better source of calycopterin (0·4% of the air dry leaves) than the copper coloured tender leaves and consequently these mature leaves could be used with advantage; further they are more easily available.

A convenient method of preparing the dimethyl ether from calycopterin using dimethyl sulphate in dry acetone medium has recently been described.[5] The synthetic sample of this substance made by the method of total synthesis[6] has also been used for the test and found to give the same result. The 4′-monomethyl ether was originally prepared by Shah,

Virkar and Venkataraman[7] by partial methylation using diazomethane. It has now been obtained more easily using dimethyl sulphate and purified by working through its potassium salt. The 5-monomethyl ether is a synthetic sample prepared by the method of Seshadri and Venkateswarlu.[8]

EXPERIMENTAL

Preparation of 4'-O-methyl calycopterin: (3:6:7:8:4'-*pentamethoxy-5-hydroxy flavone.*—A solution of pure calycopterin (1·0 g.) in a mixture of acetone (25 c.c.) and benzene (50 c.c.) was treated with dimethyl sulphate (0·27 c.c.: 1·1 mol.) and anhydrous potassium carbonate (5 g.). After refluxing for 6 hours the solvents were removed and the residue treated with water (20 c.c.). The precipitated yellow solid was filtered and washed. It was dissolved in the minimum of absolute alcohol, treated with absolute alcoholic potash (0·5 g. in 5 c.c. of absolute alcohol) and the deep red solution was evaporated to dryness quickly on a water-bath. The orange yellow solid was washed repeatedly with hot benzene to remove any dimethyl ether that might be present. The residue was treated with water acidified with hydrochloric acid, heated for a minute in a boiling water-bath and filtered. The yellow solid was crystallised from alcohol from which it

(*ge.*) = *gelatin added*

Name of the substance	Concentration in milligrams per litre	Turning time
1. Calycopterin ..	30	15·0 min.
	20	30·0 ,,
	10	73·0 ,,
2. Calycopterin dimethyl ether ..	30	11·5 ,,
	20	21·0 ,,
	10	54·0 ,,
3. Calycopterin-5-monomethyl ether ..	30, 20	More than 4 hours
	50	63·0 min.
4. Calycopterin-4'-monomethyl ether ..	5	42·0 ,,
	7	25·0 ,,
	8	14·5 ,,
	10	11·5 ,,
5. 7-Hydroxy-3-methoxy flavone ..	20	About 3 hours
	50	25·5 min.
6. Chrysin ..	20 (*ge.*)	27·5 ,,
7. Oroxylin-A ..	20 (*ge.*)	28·0 ,,
8. Gardenin methyl ether ..	20	71·0 ,,
	30	28·0 ,,
	50	12·0 ,,

separated in the form of golden yellow, stout, rhombohedral prisms melting at 126-27° (Shah *et al.*, m.p. 124°); yield, 0·6 g. It was sparingly soluble in aqueous sodium hydroxide even on heating and gave an olive green colour with alcoholic ferric chloride.

Similar procedure was adopted for preparing galangin-3:7-dimethyl ether, kæmpferol-3:7:4'-trimethyl ether and quercetin-3:7:3':4'-tetramethyl ether.

Gardenin[9] (20 mg.), galangin-3-methyl ether (20 and 30 mg.), izalpinin[10] (10 and 20 mg.), galangin-3:7-dimethyl ether (10 mg.), kæmpferol-3:7:4'-trimethyl ether (30 mg.), quercetin-3:3':4'-trimethyl ether (30 mg.), and quercetin 3:7:3':4'-tetramethyl ether were not found to be appreciably toxic. The compounds that have only one hydroxyl group in the 5-position are very sparingly soluble in water; with higher concentrations, only colloidal and non-clear solutions were obtained with the help of gelatin (1 gram for one litre of water used).

Summary

A number of partial methyl ethers of hydroxy flavones and related compounds have been tested for toxicity to fish and the results are discussed with reference to structural characteristics. Calycopterin and its 4'-methyl ether are markedly toxic.

References

1. Nadkarni .. *Indian Materia Medica*, 1927, 238.
2. Ratnagiriswaran, Sehra and Venkataraman .. *Biochem. Journal*, 1934, **28**, 1964.
3. Mahal .. *Proc. Ind. Acad. Sci.*, B, 1937, 5, 186.
4. Murti, Rao and Seshadri .. *Ibid.*, A, 1947, 25, 22.
5. Seshadri and Venkateswarlu .. *Ibid.*, A, 1946, 23, 192.
6. Murti, Rao and Seshadri .. *Ibid.*, A, 1946, 24, 233.
7. Shah, Virkar and Venkataraman .. *J. I. C. S.*, 1942, 136.
8. Seshadri and Venkateswarlu .. *Proc. Ind. Acad. Sci.*, 1946, 24, 349.
9. Bose and Nath .. *J. I. C. S.*, 1938, 139.
10. Rao and Seshadri .. *Proc. Ind. Acad. Sci.*, 1945, 22, 383.