THE CHEMISTRY OF SANTONIN

PART II[1]. PREPARATION OF SOME DERIVATIVES WITH POSSIBLE ANTHELMINTIC ACTIVITY

BY WESLEY COCKER AND T. B. H. MCMURRY

From The University Chemical Laboratory, Trinity College, Dublin

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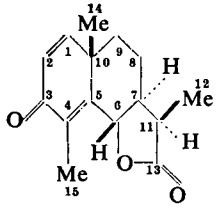
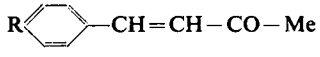
BALDWIN[2] in an excellent summary of his own and earlier work has suggested that three features of the santonin (I) molecule are essential for anthelmintic activity. They are (*a*) an intact butanolide ring, (*b*) unsaturation at C (3), and (*c*) an angular methyl group at C (10). However, inspection of the structure of many of the active non santonin-like compounds investigated by Baldwin reveals that the most active agents are those which form chelate complexes with metals. This is demonstrated by the greater activity of *o*-hydroxy- over *p*-hydroxyacetophenone, and the very great activity of 2:2′-dipyridyl and related compounds. The activity may be due to interference with an enzyme system in the nematode, by the removal of an essential metallic ion. Other promising series of compounds studied by Baldwin were the substituted benzylidene acetones (II), the phenol carbamates, and the aminothiazoles (III).



(I)



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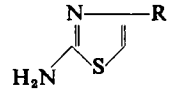


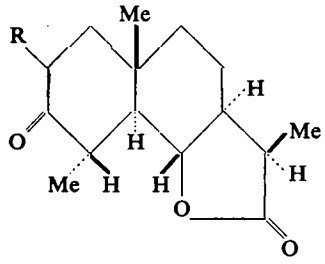
(III)

In our investigations we have applied the above principles to the santonin molecule; we have synthesised various derivatives which incorporate the three essentials mentioned by Baldwin[2] with one or more features which might be expected to increase biological activity.

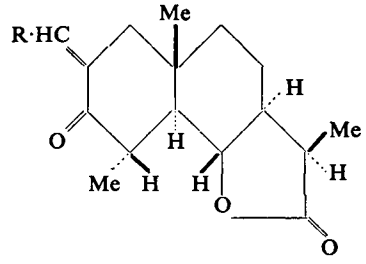
The so-called* α-tetrahydrosantonin (IV*a*) was used as starting material for our syntheses. On the basis of experiments not yet published we have tentatively assigned to it the *trans* A/B ring fusion shown[4], which is the reverse of the assignment of Yanagita and Tahara[5].

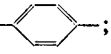
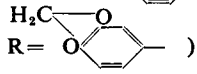
Condensation of α-tetrahydrosantonin with ethyl formate under basic conditions affords its 2-hydroxymethylene compound, which since it gives a purple ferric reaction must be (V*a*) and not (VI). The 2-benzylidene (V*b*), 2-anisylidene (V*c*), and 2-piperonylidene (V*d*) derivatives of α-tetrahydrosantonin were obtained by base catalysed condensation of this compound with the corresponding aldehyde.

* When the stereochemistry of this compound is established it will be named according to the suggestions of Cocker and Cahn[3] and Cocker and McMurry[1].

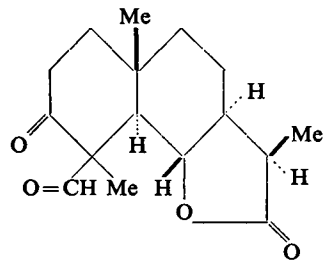


(IV; *a*, R=H; *b*, R=OH; *c*, R=OAc)

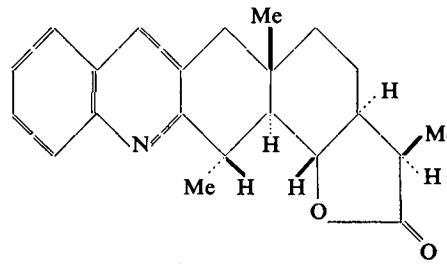


(V; *a*, R=OH; *b*, R=  ;
c, R= MeO—  ;
d, R= )

It is interesting to compare the ultra-violet absorption spectra of these condensation products with those of the corresponding benzylidene acetones (II). Fieser and Fieser[6] have shown that in the $\alpha:\beta$-unsaturated ketones substitution of an alkyl group for hydrogen in the α-position produces a batho-chromic shift of about 100 Å in the position of the low wavelength band. In the system under examination (ArCH = CR—CO—) it will be observed (Table I) that the effect of the alkyl substituent (R) diminishes as the aromatic nucleus is progressively substituted.



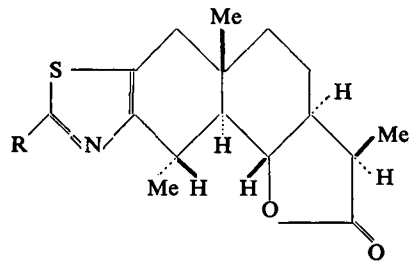
(VI)



(VII)

Condensation of α-tetrahydrosantonin with *o*-aminobenzaldehyde gave the quinoline (VII), whilst 2-bromo-α-tetrahydrosantonin[5], in which the bromine is equatorially substituted[4], when reacted with thioacetamide gave the methyl thiazole (VIII*a*).

Substitution of thiourea for thioacetamide in the last reaction gave the aminothiazole (VIII*b*) from which its acetyl derivative (VIII*c*) was obtained. The latter compound should chelate with metal ions.



(VIII; *a*, R=Me; *b*, R=NH_2; *c*, R=NHAc)

TABLE I

ULTRA-VIOLET ABSORPTION DATA OF DERIVATIVES

α-Tetrahydrosantonin derivative	$\lambda_{max.}$ Å (log ε)	Acetone derivative	$\lambda_{max.}$ Å (log ε)	$\Delta\lambda_{max.}$ Å
Benzylidene	2940 (4·29)	Benzylidene	2850 (4·35)[7]	90
Anisylidene	2320 (3·89) 3220 (4·34)	Anisylidene	2320 (4·08) 3180 (4·37)[8]	40
Piperonylidene	2500 (3·98) 3375 (4·29)	Piperonylidene	3360 (5·19)[9]	15

$\Delta\lambda$ = [$\lambda_{max.}$ (Santonin derivative) − $\lambda_{max.}$ (Acetone derivative)] is a measure of the influence of the alkyl substitutent introduced by the *cyclo*hexanone ring.

2-Acetoxy-α-tetrahydrosantonin (IV*c*) was prepared by the action of mercuric acetate in acetic acid[10] on α-tetrahydrosantonin. Hydrolysis gave 2-hydroxy-α-tetrahydrosantonin (IV*b*) which again should be capable of chelation. Yields of this compound were however too low to permit tests of its anthelmintic activity to be performed.

Preliminary screening tests carried out on the compounds described using *Ascaris lumbricoides* have so far failed to reveal any interesting degree of activity in these compounds.

EXPERIMENTAL

All ultra-violet spectra were measured by a Beckman DU spectrophotometer, using ethanol as solvent. Specific rotations were measured in chloroform. Melting points are uncorrected.

2-*Hydroxymethylene-α-tetrahydrosantonin* (V*a*). A mixture of α-tetrahydrosantonin (1·25 g.) anhydrous sodium methoxide (1·4 g.) and ethyl formate (1 ml.) in dry benzene (20 ml.) was shaken for 12 hours, and then poured on to ice and dilute sulphuric acid. The benzene layer was separated, dried and the solvent removed leaving a gum which separated from dilute ethanol as needles, thus affording 2-*hydroxymethylene-α-tetrahydrosantonin* (1·13 g.) m.pt. 143–144° C. $[\alpha]_D^{15}$ + 94·0° (c, 1·2). Light absorption: maximum 2800 Å (log ε 4·05) (in acidified ethanol). (Found: C, 68·7; H, 8·0. $C_{16}H_{22}O_4$ requires C, 69·1; H, 7·9 per cent.).

2-*Benzylidene-α-tetrahydrosantonin* (V*b*). A solution of α-tetrahydrosantonin (2·5 g.), redistilled benzaldehyde (1·06 g.) and solid potassium hydroxide (1·12 g.) in ethanol (10 ml.) was set aside for 64 hours at room temperature, during which time a deep red colour developed. The mixture was diluted with water (60 ml.), acidified with hydrochloric acid and steam distilled. The solid residue was collected and recrystallised from aqueous ethanol as pale yellow needles (2·86 g.), m.pt. 125–126° C. $[\alpha]_D^{15}$ − 131° (c, 2·64). Found: C, 77·8; H, 7·5. $C_{22}H_{26}O_3$ requires C, 78·1; H, 7·7 per cent.)

2-*Anisylidene-α-tetrahydrosantonin* (V*c*). This compound was obtained from α-tetrahydrosantonin (2·75 g.), anisaldehyde (1·25 ml.) and potassium hydroxide (1·25 g.) in ethanol (25 ml.) after standing for 48 hours. The product, 2-*anisylidene-α-tetrahydrosantonin* was obtained (3·44 g.) as

pale yellow plates (ethanol), m.pt. 195–196° C. (Found: C, 75·6; H, 7·5. $C_{23}H_{28}O_4$ requires C, 75·0; H, 7·6 per cent.)

2-*Piperonylidene-α-tetrahydrosantonin* (V*d*). α-Tetrahydrosantonin (2·5 g.), piperonal (1·5 g.) and potassium hydroxide (1·16 g.) in ethanol (15 ml.) after 48 hours gave 2-*piperonylidene-α-tetrahydrosantonin* (2·8 g.) as yellow rhombs (ethanol) m.pt. 148–149° C. $[\alpha]_D^{17}$ — 198·7° (c, 2·2). (Found: C, 71·6; H, 7·0. $C_{23}H_{26}O_5$ requires C, 72·3; H, 6·8 per cent.)

Condensation of o-*aminobenzaldehyde with α-tetrahydrosantonin.* A solution of α-tetrahydrosantonin (0·65 g.), o-aminobenzaldehyde (0·35 g.), and sodium hydroxide (1·35 g.), in ethanol (12 ml.) and water (8 ml.) was kept for 64 hours at room temperature. It was diluted with water, extracted with ether, and acidified with hydrochloric acid. The volume was reduced to 5 ml., and the mixture was carefully neutralised with sodium hydroxide. The quinoline derivative (VII) was deposited and it was crystallised from ethanol as needles (0·82 g.), m.pt. 263° C. Light absorption: maxima, 2325, 3075, 3210 Å (log ϵ 4·85, 3·73, 3·85 respectively); *cf.* 2-methylquinoline[11] which shows maxima at 2740, 3150 Å (log ϵ 3·54, 3·6 respectively). (Found: C, 78·2; H, 7·1. $C_{22}H_{25}O_2N$ requires C, 78·8; H, 7·5 per cent.)

The quinoline (0·2 g.) failed to yield a methiodide after refluxing with methyl iodide (2 ml.) for 30 minutes.

Methylthiazole (VIII*a*). A solution of 2-bromo-α-tetrahydrosantonin (0·65 g.) and thioacetamide (0·2 g.) in pyridine (2 ml.) was heated on the water bath for 45 minutes. The mixture was poured into water and the solid (0·45 g.) collected. After treatment with charcoal in boiling benzene the product was recrystallised from ethanol as rhombs, m.pt. 234° C. Light absorption: maximum 2530Å (log ϵ 3·64). *Cf.* Thiazole[12] which shows a single maximum at 2400 Å (log ϵ 3·60). (Found: C, 67·0; H, 7·5. $C_{17}H_{23}O_2NS$ requires C, 66·9; H, 7·5 per cent.)

Aminothiazole (VIII*b*). A mixture of 2-bromo-α-tetrahydrosantonin (0·65 g.), thiourea (0·20 g.) and pyridine (2 ml.) was heated on the water bath for 45 minutes. The mixture was poured into water, and the solid (0·51 g.) collected, decolourised with charcoal in benzene solution and recrystallised from ethyl acetate, from which the *aminothiazole* was obtained as rhombs, m.pt. 271–272° C. Light absorption: maximum 2630° Å (log ϵ 3·80); *cf.* 2-aminothiazole which gives a maximum at 2520 Å (log ϵ 3·8)[13]. (Found C, 62·1; H, 7·0. $C_{16}H_{22}O_2N_2S$ requires C, 62·7; H, 7·2 per cent.)

Acetamidothiazole (VIII*c*). A mixture of the aminothiazole (VIII*b*) (0·2 g.) and acetic anhydride (2 ml.) was heated for 1 hour at 100° C. The solution was poured into water and the acetic acid neutralised with sodium hydrogen carbonate. The solid (0·21 g.) was collected and recrystallised from ethanol as needles, m.pt. 308–309° C. Light absorption: maximum, 2790 Å (log ϵ 4·04). (Found: C, 62·0; H, 6·8. $C_{18}H_{24}O_3N_2S$ requires C, 62·1; H, 6·8 per cent.)

2-*Acetoxy-α-tetrahydrosantonin* (IV*c*). A mixture of α-tetrahydrosantonin (1·25 g.) and mercuric oxide (1·17 g.) in glacial acetic acid (15 ml.) was refluxed for 2·5 hours. A solid rapidly separated and then

slowly dissolved. At the end of the reaction the solution was decanted from mercury, diluted with water, neutralised with sodium hydrogen carbonate, and the resulting gum collected in chloroform. The gum was dissolved in ethanol, and allowed to stand overnight when a solid (0·4 g.) was deposited. It was decolourised by charcoal in benzene solution, and recrystallised from ethanol as needles, m.pt. 173–174° C. (Found: C, 65·8; H, 7·8. $C_{17}H_{24}O_5$ requires C, 66·2; H, 7·8 per cent.)

2-Hydroxy-α-tetrahydrosantonin (IV*b*). A solution of 2-acetoxy-α-tetrahydrosantonin (0·4 g.) and potassium hydroxide (0·6 g.) in methanol (30 ml.) was refluxed for 2 hours. The solvent was removed, the residue was acidified with hydrochloric acid, and the mixture was extracted with chloroform. The extract was shaken several times with sodium hydroxide solution, and the combined alkaline extracts were acidified and extracted with chloroform. The resulting gum gave a positive ferric reaction, and on extraction with ethanol gave the required compound (0·23 g.) as needles, m.pt. 157–158° C. (Found: C, 67·6; H, 7·6. $C_{15}H_{22}O_4$ requires C, 67·7; H, 8·3 per cent.)

α-Tetrahydrosantonin thiosemicarbazone. A mixture of α-tetrahydrosantonin (1·0 g.), thiosemicarbazide (1·0 g.) and fused sodium acetate (0·4 g.) in methanol (25 ml.) was refluxed for 6 hours. The solvent was removed, the solid residue was washed with water and crystallised from ethanol from which the *thiosemicarbazone* (1·05 g.) separated as needles, m.pt. 234° C. (Found: C, 59·1; H, 7·8. $C_{16}H_{25}O_2N_3S$ requires C, 59·4; H, 7·8 per cent.)

α-Tetrahydrosantonin semicarbazone. This compound had m.pt. 236–237° C. (rapid heating). Weinhaus and Oettingen[14] record m.pt. 256–258° C.

Summary

1. A number of derivatives of santonin incorporating the β-methyl group at C (10), the ketonic group or its equivalent at C (3), and the intact butanolide fused at C (6)–C (7) to the bicyclic system have been synthesised.

2. These derivatives were related to non santonin-like compounds which have anthelmintic activity.

3. The combination of features of the santonin molecule might reasonably have been expected to produce activity. Preliminary screening tests have unfortunately shown no promise of such activity.

The authors gratefully acknowledge the financial support given by Messrs. T. and H. Smith, Ltd. of Edinburgh and the Medical Research Council of Ireland.

References

1. Part I. Cocker and McMurry, *J. chem. Soc.*, 1955, 4430.
2. Baldwin, *Brit. J. Pharmacol.*, 1948, **3**, 91.
3. Cocker and Cahn, *Chem. and Ind.*, 1955, 384.
4. Cocker and McMurry, Unpublished work.
5. Yanagita and Tahara, *J. org. Chem.*, 1955, **20**, 959.
6. Fieser and Fieser, *Natural Products related to Phenanthrene*, Reinhold Publishing Co., New York, 1949, p. 190.

7. Lowry, Moureu and MacConkey, *J. chem. Soc.*, 1928, 3167.
8. Wilds, Beck, Close, Djerassi, Johnson, Johnson and Shunk, *J. Amer. chem. Soc.*, 1947, **69**, 1985.
9. Herzog and Hilmer, *Ber.*, 1931, **64**, 1388. (The log ϵ value quoted here is probably not reliable.)
10. Triebs and Bast, *Ann.*, 1948, **561**, 165.
11. Sutherland and Compton, *J. org. Chem.*, 1952, **17**, 1257.
12. Braude, *Ann. Repts.*, 1945, **42**, 128; Ruehle, *J. Amer. chem. Soc.*, 1935, **57**, 1887.
13. Vandenbelt and Doub, *ibid.*, 1944, **66**, 1633.
14. Weinhaus and Oettingen, *Ann.*, 1913, **397**, 219.

DISCUSSION

The paper was presented by MR. T. B. H. MCMURRY.

DR. G. E. FOSTER (Dartford) said that santonin became discoloured very readily in light. He wondered whether any of the compounds which the authors had made possessed similar photochemical properties, and if so were they able to relate these properties to the structure.

DR. W. MITCHELL (London) said he was puzzled by the opening paragraph where reference was made to the fact that certain active compounds could chelate metals. Immediately afterwards reference was made to compounds II and III which could not, so far as he could see, chelate metals. It would be interesting to know whether compound Va could chelate metals.

DR. A. H. BECKETT (London) suggested that the chelation of trace metals was a vital factor. It was amazing to observe the avidity with which bacteria could compete with chelating agents.

MR. T. B. H. MCMURRY, in reply, said that form IVa did not discolour in light. This change was associated with double bonds and the ketone groups in santonin. With regard to chelation, the statement was based on the fact that of the compounds which were examined by Professor Baldwin only 2:2'-dipyridyl and related compounds had the same activity as santonin. The other compounds examined were not nearly as active. 2:2'-Dipyridyl was too soluble for use: it was quickly eliminated from the system. Compound Va will chelate, and one can obtain a very good reaction with ferric iron.

PROFESSOR W. COCKER added that the testing of the materials was entirely out of the authors' hands.