Tetrahedron. Vol. 27. pp. 3051 to 3063. Pergamon Press 1971. Printed in Great Britain

BIOGENETIC-TYPE SYNTHESIS OF POLYKETIDES. PART X

SYNTHESIS AND REACTIONS OF HEPTA AND NONA-β-CARBONYL CHAINS AS SUBSTRATE MODELS

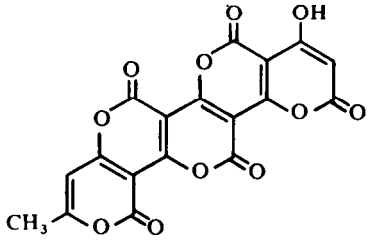
A. I. SCOTT, D. G. PIKE, J. J. RYAN and H. GUILFORD
Kline Chemistry Laboratory, Yale University, New Haven, Conn.

(*Received in USA* 5 *November* 1970; *Received in the UK for publication* 11 *November* 1970)

Abstract—A method for the synthesis of extended poly-β-carbonyl chains has been developed in which control of cyclization and reductive sequences paralleling those of nature are made possible. The symmetrical ketone **7** becomes the key compound in these studies which incidentally led to the preparation and characterization of **23**, a derivative of penta-acetic acid.

IN previous papers from our laboratory[1–9] and elsewhere[10–18] synthetic routes have been studied for the construction of aromatic metabolites of polyketide origin *via* intermediates reminiscent of the postulated precursors of Birch's polyacetate hypothesis.

So far our experiments have led to the linear *tetra*, *penta* and *hexa*-acetate systems and their corresponding aromatized derivatives. Although the pentapyrone (**1**)

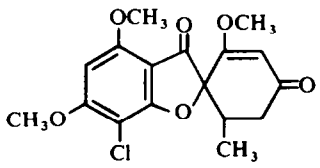


1

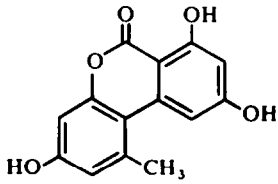
containing an array of seven such units recently became available,[9] our experience with the condensed pyrones indicated that, in view of the small yields attainable for the synthesis of more complex members, alternative methods for the higher homologues would have to be sought.

There appear to be four essential requirements for successful substrate model studies related to the biosynthesis of such complex metabolites as griseofulvin (**2**), griseoxanthone C (**3**) and alternariol (**4**) which contain seven "acetate" residues; endocrocin (**5**) (octaacetate); and (at the probable experimental limit) tetracycline precursors, such as methylpretetramid (**6**). These requirements, which correspond to the presumed[10] biochemical events are summarized as (A): construction of seven to ten alternating

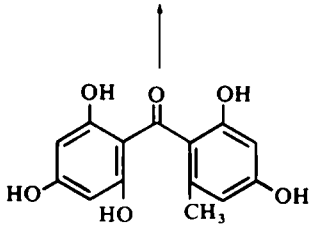
* Support for this work is gratefully acknowledged as follows: 1962–65 N.R.C. (Canada); 1965–68 USPHS and S.R.C. (U.K.); 1968–70 USPHS Grant AI-08920 and the Sandoz Foundation.

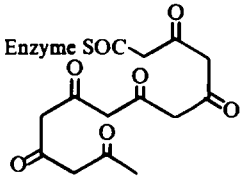


2

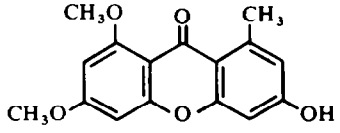


4

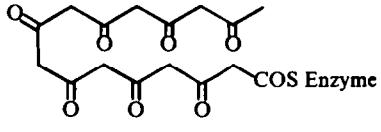




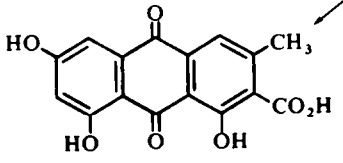
"Heptaketide"



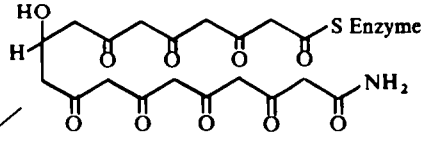
3



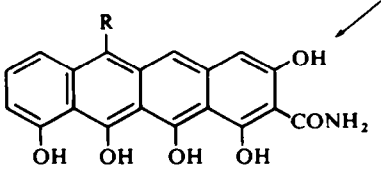
"Octaketide"



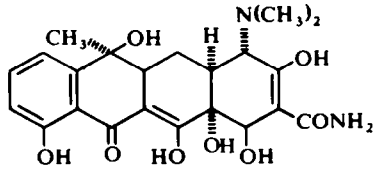
5



"Malonamyl octaketide"



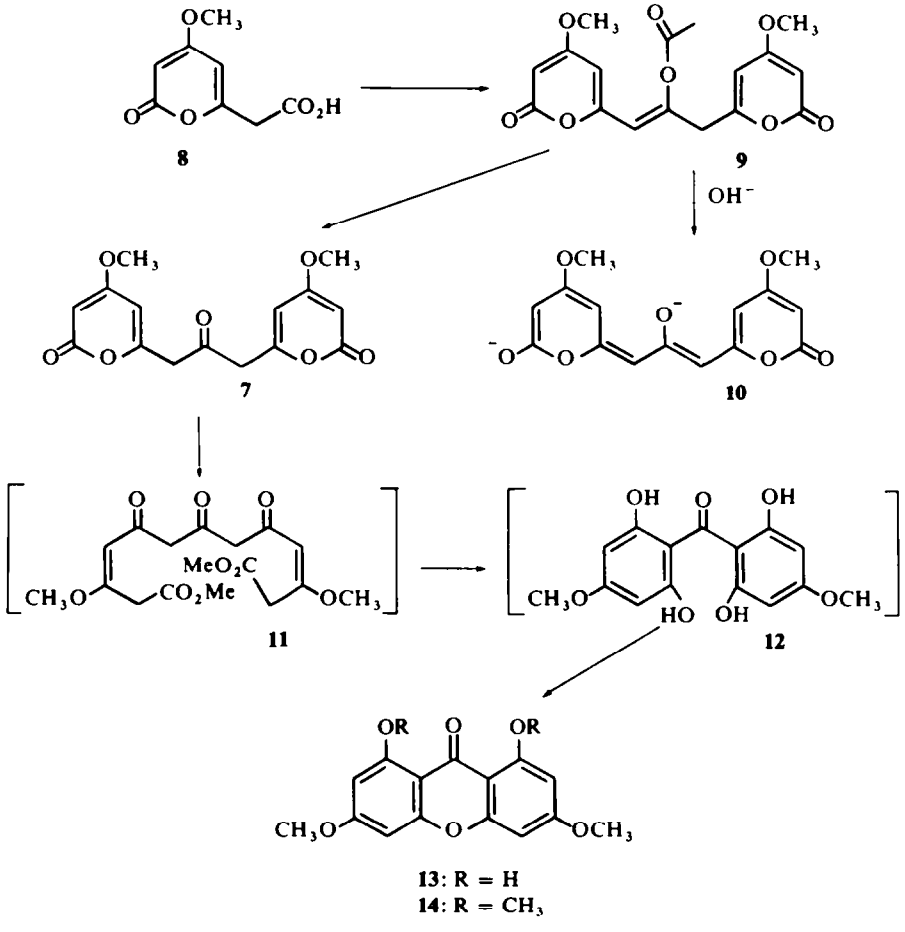
6 R = CH_3



Tetracycline

linear carbonyl (or equivalent) functions; (B) Control of cyclization mechanism (e.g. **2** and **4** are formed from the same chain by different mechanisms); (C) Selective reduction of those carbonyl groups on the chain which correspond to the site of biochemical reduction (e.g. **6**); (D) Control of sequence of ring closure for more extended systems (e.g. **5**, **6**) where many possibilities obviously exist.

Our earlier studies have established partial solutions for (A) and (B) but the recognised limitations inherent in these primitive models led us to design a new set of substrates. The first molecule of choice for these experiments was the symmetrical



ketone (**7**) which became readily available by decarboxylative dimerization of the acid (**8**).[19] The masked β-polycarbonyl is theoretically capable not only of meeting the first two requirements but also offers hope in solving problems C and D. We now consider in turn the preparation of hepta- and nona-β-carbonyl chains based on the chemistry of (**7**).

The hepta-β-carbonyl series.

Treatment of the acid (**8**)[19] with acetic anhydride-triethylamine afforded the enol acetate (**9**) in 55% yield. The structure (**9**) followed from analytical and spectral data. In particular the NMR spectrum ($CDCl_3$) showed signals at τ 7·68 (3H, s); 6·53 (2H, s), 6·21 (3H, s); 6·19 (3H, s); 4·53 (2H, d, J = 2 Hz); 4·05 (2H, d J = 2Hz), 4·20 (1H, s). Addition of base effected a shift in the UV spectrum from 310 nm to λ 275, 390, 572 nm corresponding to the generation of the magenta-coloured di-anion (**10**). From aqueous basic treatment of **9** the desired *symmetrical ketone* (**7**) λ_{max}283 nm could be isolated and exhibited the expected NMR signals viz. CH_2 at τ 6·33 (4H, s), OCH_3 at 6·18 (6H, s) and pyrone ring protons at 4·52 (2H, d, J = 2 Hz) and 4·03 (2H, d, J = 2Hz).

In meeting requirements A and B the symmetrical ketone (**7**) proved adequate, for not only could the latent carbonyl functions be revealed, but the presence of the protective methyl ether groupings at $\underline{C}_3$ and $\underline{C}_{11}$ in the derived poly-β-carbonyl (**11**) were so arranged as to dictate the ring closure mechanism. When reduced to laboratory practice this system constituted a source of the polyhydroxyanthone series. Thus, in the spectrum of a methanolic solution of **7** containing NaOMe the long wavelength band (λ 392 nm) slowly disappeared. Acidification and isolation gave a 15% yield of 1.8-dihydroxy-3.6-dimethoxyxanthone (**13**) formed presumably by methanolysis (**7**) → (**11**) and Claisen condensation to the fugitive intermediate, 2,6,2′,6′-tetrahydroxy-4,4′-dimethoxy-benzophenone (**12**). The structure of the xanthone (**13**) was confirmed by conversion to 1,3,6,8-tetramethoxyanthone (**14**) and comparison with an authentic sample.[20] The absence of any detectable product of aldol condensation in this reaction is testimony to the controlling influence of methyl ether functionality in a particular environment.* The oxygenation pattern of xanthone (**13**) does not correspond exactly to a xanthone of pure polyketide origin derived from a "head-to-tail" precursor as shown for **3**. However the success of this experiment indicated possible extension to more rigorous models and to higher homologues. Thus the main difference between the hepta-β-carbonyl system (**11**) and the presumed enzyme bound precursor of griseoxanthone C (**3**) and of alternariol (**4**) is the substitution of OCH_3 for CH_3 in the acetate starter unit. A further point of interest is the presence of enol ether functions in **11** which serve as a substitute or perhaps even a model for enzymic control of cyclisation *via* enol formation.

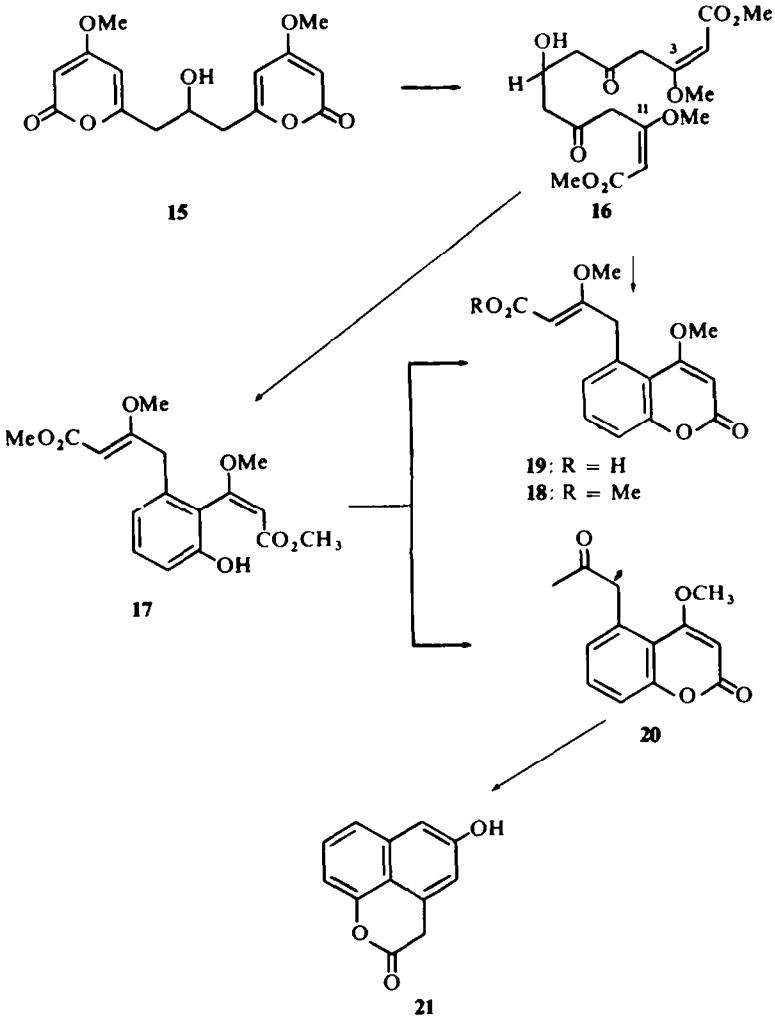
Before proceeding to longer chain lengths we examined a model for requirement C by reducing the ketone (**7**) to the secondary alcohol (**15**) in high yield, using $NaBH_4$. The alcohol exhibited spectral data in full accord with structure (**15**) and, in contrast to the ketone (**7**), gave no wavelength base shift in the UV spectrum. Thus the methylene groups in 15 display diminished reactivity by comparison with **7**. We therefore expected to find a different type of aromatization reaction of the methanolyzed species (**16** → (**17**). A solution of **15** in methanolic KOH was acidified after 2 hr and a complex mixture obtained from which three closely related aromatic products (**18–20**) could be isolated (TLC) and characterized.

The least polar of these, compound **18** displayed λ_{max}230, 273 and 283 nm and in the NMR revealed 3 methyl, 2 vinyl, 1 methylene and 3 aromatic proton signals. This information, combined with a molecular ion peak at m/e 304 in the mass spectrum, suggested that the structure is in fact the alkylated coumarin (**18**). Although the UV,

* The efficiency of this method of control is not however sustained in all cases.[21]

and IR data leave no doubt that the 4-methoxy coumarin chromophore is present, the signal at 5·34 τ assigned to CH_2 adjacent to the aromatic ring and also in an allylic position is at somewhat lower τ value than expected. The effect is however explicable on the grounds of deshielding by 3 neighbouring oxygen functions.

Structure **19** follows for the second, more polar product since it is readily converted back to **18** by diazomethane treatment.

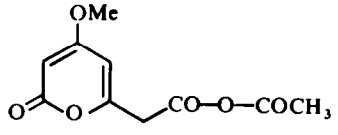


The last product (**20**) is ketonic in nature and contains in addition to a 4-methoxy-coumarin chromophore the system Ar-CH_2COCH_3[τ 5·88 (2H, s) and 7·80 (3H, s)]. Since this ketone could also be formed by treatment of (**18**) with methanolic HCl, structure (**20**) can be assigned.

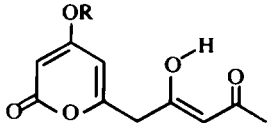
The ubiquitous formation of the 5-substituted coumarin pattern from the secondary

alcohol (**15**) seemed at first sight rather disappointing for it was hoped that naphthalenoid structures would have emerged from the reactions of the intermediates **16** and **17**. However, with the exception of a small amount of a compound believed to be **21** (see Experimental) formed by vigorous base treatment of (**20**) the stability of the 4-methoxycoumarin moiety dominated the chemistry of these compounds. Nevertheless the genesis of **18–20** via **16** and **17** is illustrative of the solution to conditions C and D. Modifications at C_{11} of **16** are obvious starting points for directing the closure towards naphthol rather than hydroxycoumarin structures. Also, the loss of the alcohol function in the aromatization reaction at once provides a model for those polyketides which lack an oxygen function in a position, which does not necessarily direct the ring closure reaction, except perhaps by the dehydrative genesis of a *cis*-olefin prior to ring formation.

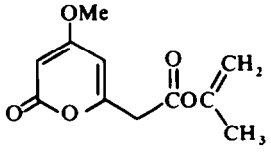
During exploratory experiments on the synthesis of **7** an attempt was made to prepare the mixed anhydride (**22**) of the acid (**8**) using the procedure of Hagenmeyer and Hull.[22] In one of these experiments it was found that heating a solution of **8** in isopropenyl acetate containing a few drops of H_2SO_6 led to a 37% yield of crystalline



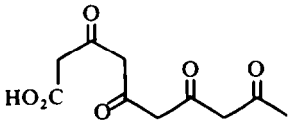
22



23: R = Me
24: R = H



25



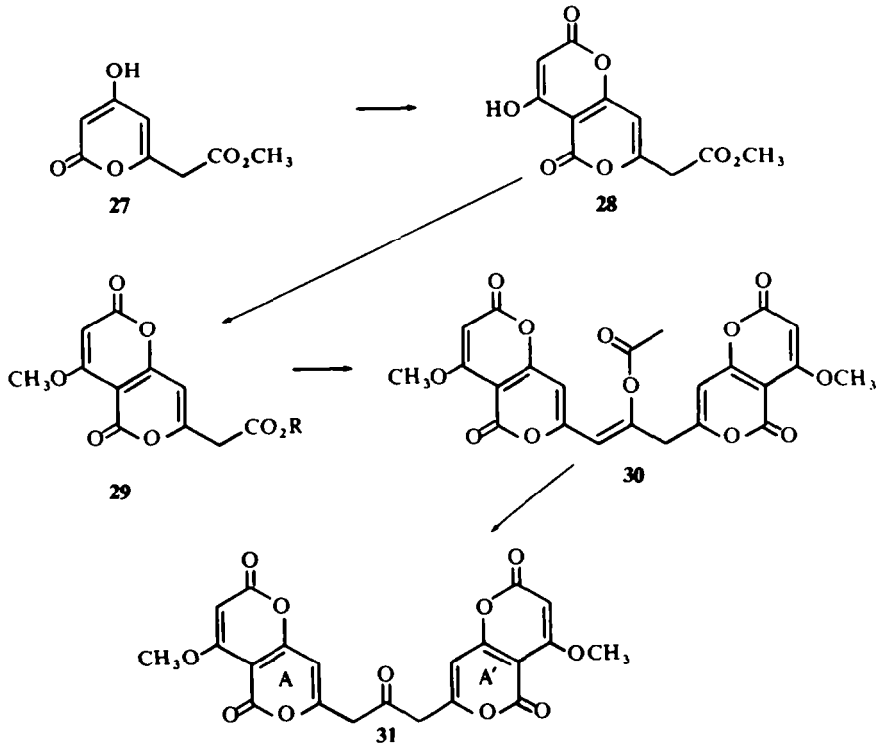
26

monopyrone $C_{11}H_{12}O_5$ m.p. 92–3° whose structure (**23**) is derived from the following spectroscopic data: λ_{max} 287 nm (ε 15.200) changed in base to 298 and 394 nm (ε 22.000. and 1.500 respectively) and suggesting an extensively conjugated enolised system; ν 1740 (>C=O of pyrone) 1725 (>C=O) 1625 (H bonded >C=O) cm^{-1}; τ 7·93 ($C\underline{H}_3$, s), 6·58 ($C\underline{H}_2$, s), 6·18 (OCH_3, s), 4·52 (H_2, d; J = 2 Hz), 4·40 H_3, s, 4·03 H_2 d J = 2 Hz; −3 → −6 (OH broad); and *m/e* 224, 182, 166 140 (base peak) and 125. Structure **23** is the methyl ether of the homologue of the naturally occurring tetraacetic lactone[23] and therefore represents a reference compound of some value in future biosynthesis studies, although the isolation of the corresponding 4-hydroxy pyrone, pentaacetic lactone (**24**) is still awaited. A possible intermediate in this reaction is the isopropenyl ester (**25**). However all attempts to rearrange this ester have so far failed to produce any of the desired diketone (**23**). Since great difficulty was also experienced in reproducing yields of greater than 10% of the pyrone (**23**) using varying amounts of

acidic catalysts, this approach has been set aside pending the anticipated isolation of this masked form of pentaacetic acid (**25**).

Nona-β-carbonyl series.

Turning now to the synthesis of the nona-β-carbonyl system it was found possible to apply the "symmetrical ketone approach" to the preparation of the dipyrone ketone (**31**). This followed established procedures developed in our laboratories, the route being depicted in Scheme 1. As in the case of the simpler symmetrical ketone (**7**) self condensation of the dipyrone acid (**29**; R = H) gave crystalline enol acetate (**30**) and in fact this was used as a convenient source of the ketone (**31**) for all experiments



SCHEME 1

in basic solution. Within the structure (**31**) resides a potential solution to *all four* of our original requirements which were originally imposed by a quest for biochemical analogy. Thus:

(A) The structure **31** contains nine alternating carbonyl functions.

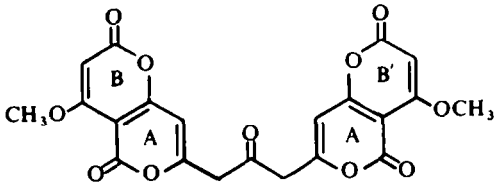
(B) Control of cyclization mechanism has been previously achieved for condensed pyrones with up to three contiguous rings.

(C) Selective reduction of the central ketonic function has already been utilised with the model (**7**).

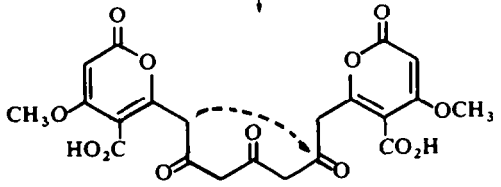
(D) Most importantly for our model reaction, the *sequence* of hydrolytic ring opening of the dipyrone system has been studied in sufficient detail for us to anticipate

that rings A and A′ would be simultaneously and selectively cleaved in basic solution leading to the reactive intermediate **32**, and hence to a control of ring closure sequence reminiscent of the specificity of pretetramid biosynthesis (*cf.* the genesis of **6**).

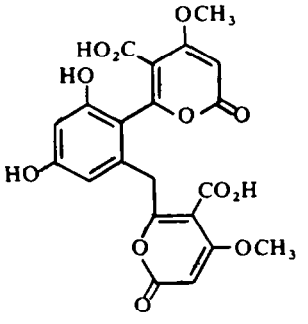
With three proven requirements inherent in the symmetrical ketone (**31**) it remained to test the operation of the fourth condition. We were gratified to find that the colourless acetate (**30**) when dissolved in aqueous alkali immediately formed a deep blue solution λ_{max}648 nm to which we ascribe a dianionic structure (**30a**). The reversibility of the formation of this ion was sustained in alkaline solution for 30 min.



31

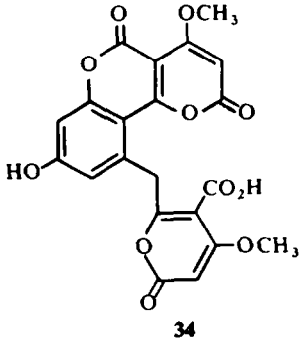


32

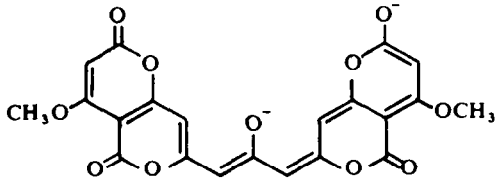


33

—H_2O



34

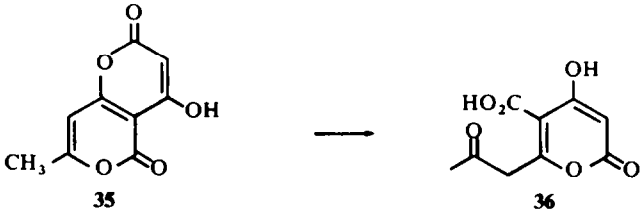


30a 648 nm

For preparative purposes the reaction of (**30**) with NaOH aq was monitored spectroscopically by observation of the absorption at λ_{max}648 nm. When this absorption had diminished to less than 1% of its original intensity the reaction was acidified to give a light brown precipitate. This was isolated and found to have ultraviolet absorption at λ_{max}438, 302, 270 and 213 nm which on acidification changed to λ_{max}320, 265, 211 nm. The NMR spectrum in CF_3COOH was weak due to lack of solubility but appeared to be rather simple with signals at 5·87 τ, 3·98 τ and 3·23 τ in the ratio 8 : 2 : 2. The IR spectrum showed bands at ν_{max}3440 (broad), 3100, 3000 to 2800 (several; suggesting carboxylic acid), 1730, 1640, 1590 and 1540 $cm.^{-1}$ This new compound appeared to be rather involatile in the mass spectrometer but peaks at *m/e* 442 and 398 were observed at very low intensity. Smaller fragments at *m/e* 110, 85 and 69 indicated the presence of an α-pyrone. Examination of the possible products that would be formed assuming preferential hydrolysis of rings A and A′ (see **28**) led to the formulation of **33** or **34** as a possibility for the hydrolysis product.

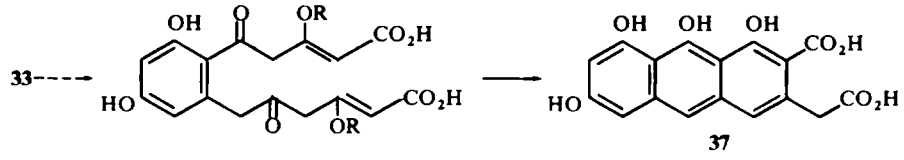
The sequence of opening of the pyrone rings in this system (A > B) finds analogy in the synthesis of 5-carboxy-tetraacetic acid lactone (**36**) from dipyrone (**35**) by treatment with KOH aq.

As indicated above the physical data for this product can be explained on the basis of structure **33** or **34**. The IR spectrum shows the bands expected for the carboxylic acid and hydroxyl at 2800 to 3000 cm^{-1} and 3100 and 3440 cm^{-1}, as well as bands typical of α-pyrones. The NMR spectrum can be explained by coincidence of the signals due to the methoxyls and methylene at 5·8τ. The signal at 3·98τ is probably due to the pyrone ring protons and that at 3·23τ to the aromatic protons. The mass spectral peak at *m/e* 442 either represents the molecular ion, in which case structure **34** is favoured, or the loss of water from molecular ion at *m/e* 460, required by structure **33**.



Attempts were made to methylate the acid with CH_2N_2 and $(CH_3)_2\,SO_3$[24] but no reaction could be detected, probably due to the insolubility of the starting material. Attempts to silylate the compound with trimethylsilyl chloride or hexamethyldisilazane failed to give products that were volatile in the mass spectrometer.

The compound was treated with refluxing NaOH aq yielding material which had UV absorption at λ_{max}305, 225 (sh) and 211 nm. The mass spectrum of this substance was very weak, the highest peak being observed at *m/e* 168, indicating that under the vigorous conditions of the reaction, degradation had occurred.



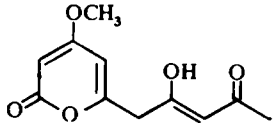
37

Although the anthrol (**37**) was not detected, the isolation of **33** shows that the nona-β-carbonyl system exemplified by **31** does not undergo complete degradation on treatment with base. In summary the stratagems developed in the course of this work has allowed the construction of a masked nona-β-carbonyl array in which selectivity with respect to both hydrolysis and subsequent ring closure has been embedded. The ultimate goal of this approach, pending the identification of poly-β-carbonyls from natural systems is the preparation of the tetracycline or pretetramid nucleus (**6**) in a manner reminiscent of the postulated biochemistry. As a result of these model experiments a full-scale attack on the synthesis of complex natural phenols can be mounted.

EXPERIMENTAL

Preparation of pentaacetic acid lactone methyl ether (**23**). 6-Carboxymethyl-4-methoxy-2-pyrone (460 mg) was stirred with isopropenyl acetate 2 ml and conc. H_2SO_4 (3 drops) in a flask fitted with a Vigreux column and a distillation receiver. The mixture was heated in an oil bath at 70–90°C but no acetone distilled. The flask was then allowed to cool and stand and the mixture was distilled, 25°C 0·5 mm. The colourless distillate collected was isopropenyl acetate.

The residue was a red, $CHCl_3$-soluble tar. This was chromatographed on silica gel, first with $CHCl_3$ (1 l.) and then with 2% MeOH in $CHCl_3$, and run dry in 10% MeOH/$CHCl_3$. The $CHCl_3$ fractions gave 210 mg of a solid which crystallized from $CHCl_3$ ether at −17° to give pale yellow needles m.p. 88–90°. An analytical sample m.p. 92–93° recrystallized from $CHCl_3$:petroleum ether. UV λ_{max} 287 nm (ε 15200); λ_{max} (base) 298 nm (ε 22000), 394 (ε 1500) nm. IR ν_{max} 3250–3500 (br, m), 1740 (sh, m), 1725 (s), 1625 (m), 1570 (m).



23

NMR ($CDCl_3$) 7·93, $3H_a$(s); 6·58, $2H_b$(s); 6·18, $3H_c$(s); 4·52, $1H_d$(d) J = 2 cps; 4·40, $1H_e$(s); 4·03, $1H_f$(d) J = 2 cps; −3·3 to −6·7, $1H_g$(br). MS M^+ 224 *m/e* 182, 166, 140 (B^+), 125, 112, 97, 85, 60, 43. $FeCl_3$ test: (weak), pale orange complex. ($C_{11}H_{12}O_5$ (224.2) requires C, 58·92; H, 5·79. Found: C, 58·65; H, 5·86%). Yield 37%.

Preparation of the symmetrical monopyrone enol acetate (**9**). The pyrone acid (**8**, 400 mg) was dissolved in pure, dry THF (20 ml). Redistilled acetic anhydride (2 ml) followed by triethylamine (2 ml, Na dried) was added to the stirred solution at room temperature. After 30 min the solution was poured into distilled H_2O (30 ml) and the aqueous solution extracted with $CHCl_3$ (3 × 50 ml). The extracts were combined and extracted with an 150 ml. of 2M NaOH aq., washed with distilled H_2O, dried over Na_2SO_4 and evaporated yielding a pale yellow oil. This was dissolved in MeOH (10 ml) and distilled H_2O (75 ml) added. On standing for 15 min crystals formed; there were filtered and recrystallized from acetone/H_2O (1:3) yielding white needles (160 mg, 42%). M.p. 165–166°C. UV λ_{max}^{MeOH} 312 nm (ε 11·000), 22 nm (ε 21·500). IR (KBr) ν_{max} 1760, 1730, 1710, 1650, 1620 cm^{-1} NMR ($CDCl_3$) τ 7·68 (s, 3H), 6·19 (s, 6H), 4·53 d (J = 2 Hz, 2H), 4·2 (1H, s),

4·05 d (J = 2 Hz, 2H). MS m/e 348 (M^+) 306 (M—CH_2=C=O; 167b, 140, 125, 111, 69. Metastables m/e 268·5 (348–306), 91·2 (306–167). ($C_{17}H_{16}O_8$ requires, C, 58·62; H, 4·63. Found: C, 58·79; H, 4·64%).

Preparation of symmetrical ketone (**7**). The pale yellow oil obtained from evaporation of the $CHCl_3$ extract in the preparation of the enol acetate (**8**) was dissolved in MeOH (15 ml) and the solution made basic by the addition of 2M NaOH aq. After 2 min the solution was adjusted to pH 6–7 by the addition of 2M HCl. The solution was reduced in volume to *ca.* 5 ml and distilled H_2O (40 ml) added. The mixture stood overnight, during which time white crystals formed, (162 mg, 48%). An analytical sample was recrystallized from acetone/H_2O (1:3) to give colourless needles. M.p. 194–195°C. UV λ_{max}^{MeOH} 282 nm (ε 13·100), 208 nm (ε 31·050). IR (KBr) ν_{max} 1730, 1720, 1660, 1580 cm^{-1}. NMR ($CDCl_3$) τ 6·35 (4H, s), 6·20 (6H, s) 4·53 (d, J = 2 Hz, 2H), 4·02 (d J = 2 Hz 2H). MS m/e 306 (M^+) 167, 140, 125b, 111, metastable at m/e 91·2 (306–167). ($C_{15}H_{14}O_7$ requires C, 58·82; H, 4·61. Found: C, 58·80; H, 4·39%).

Ring opening of symmetric ketone (**7**). The symmetric ketone (**7**, 200 mg) was dissolved in 1M methanolic KOH (50 ml) and stirred under N_2 for 17 hr. The solution was cooled in an ice-bath and acidified with HCl to pH 7. The MeOH was evaporated and H_2O (60 ml) added. The resultant crystals were almost pure 1,8-dihydroxy-3,6-dimethoxyxanthone (**13**, 27 mg, 15%). M.p. 193–194°C. UV λ_{max}^{MeOH} 327 nm (ε 21·000), 270 nm (ε 8·200), 251 nm (ε 35·000), 234 nm (ε 21·000), 211 nm (ε 20·500). IR (KBr) ν_{max} 1700, 1650, 1630, 1610 cm^{-1} NMR ($CDCl_3$) τ 6·13s (3H), 3·65 (J = 2·5 Hz, 2H, AB quartet; $\nu_0\delta$ 2·4 Hz); −2·0, 1H – exchanges with D_2O. MS 288 (M^+) 182, 105, 91, 77. ($C_{15}H_{12}O_6$ requires C, 62·50; H, 4·23; Found: C, 62·47; H, 4·20%).

Methylation of 1,8-*dihydroxy*-3,6-*dimethylxanthone* (**13**). 1,8-Dihydroxy-3,6-dimethylxanthone (13, 5 mg), anhyd. K_2CO_3 carbonate (200 mg) and $(CH_3)_2SO_4$ (0·1 ml) were refluxed in dry acetone (10 ml) for 6 hr. The acetone was evaporated, distilled H_2O (5 ml) added and recovered with $CHCl_3$. The product was purified by PLC (0·5 mm silica, C_6H_6/MeOH/AcOH acid, 45:8:4) yielding white needles (3 mg, 55%) of 1,3,6,8-tetramethoxyxanthone (**14**). M.p. and m.m.p. with an authentic sample 219–220°C. UV λ_{max}^{MeOH} 305 nm, 274 nm, 246 nm, 240 nm (sh), 211 nm. IR (KBr) ν_{max} 1650 cm^{-1}, 1615, 1575 cm^{-3}. MS 316 (M^+).

Sodium borohydride reduction of symmetric ketone (**7**). The symmetric ketone (655 mg) was dissolved in 10% MeOH aq (70 ml) and a solution of $NaBH_4$ (250 mg) in 10% MeOH aq (30 ml) added. The mixture was stirred for 30 min. at room temperature and filtered yielding the alcohol (**15**, 413 mg). Distilled H_2O (10 ml) was added to the filtrate and the solution acidified to pH 0 with 2M HCl (4 ml). The MeOH was evaporated and the solution cooled yielding a further quantity of the alcohol (147 mg, 87% yield overall). Recrystallization from EtOH gave white crystals. M.p. 219–220°C. UV λ_{max}^{MeOH} 282 (ε 12·000), 208 (ε 30·000) nm. IR (KBr) ν_{max} 3400 (m), 1730 (s) 1680 (s), 1650 (s) cm.$^{-1}$ NMR ($CDCl_3$) τ 6·95 (d, J = 6 Hz, 4H), 5·98 (s, 6H), 5·18 (t J = 6 Hz, 1H), 3·96 (d, J = 2 Hz 2H), 3·51 (d, = 2 Hz 2H). MS m/e 308 (M^+) 290, 217, 205, 169, 140, 125b, metastables at m/e 92·6 (308–169) and 63·6 (308–140). ($C_{15}H_{16}O_7$ requires C, 58·44; H, 5·23; Found: C, 58·43; H, 5·31%).

Ring opening of symmetric alcohol (**15**). The symmetric alcohol (**15** 1·33 g) was dissolved in 1 M meth anolic KOH (450 ml) under N_2 and stirred at room temperature for 2 hr. The MeOH was evaporated to 100 ml and the cooled solution acidified to pH 0 with 2N HCl (200 ml). The aqueous solution was extracted with $CHCl_3$ and EtOAc. The pH was adjusted to pH 7 and the aqueous solution re-extracted. The organic solvents were combined, dried (Na_2SO_4), filtered, evaporated, and the residue triturated with $CHCl_3$. The $CHCl_3$ was filtered and evaporated yielding a complex mixture (716 mg). The mixture was separated on preparative TLC using 0·75 mm silica developed with $CHCl_3$/acetone 9:1. The following bands were isolated, eluted with acetone and evaporated,

(i) R_f 0·8 coumarin A (**18**) white needles from EtOAc (21 mg). M.p. 146–148°C· UV λ_{max}^{MeOH} 305 (sh) (ε 5·000), 284 (ε 14·000), 273 (ε 15·000), 230 (ε 24·000), 217 (ε 29·200) nm. IR ($CHCl_3$) ν_{max} 1700 (s), 1605 (s) cm^{-1} NMR ($CDCl_3$) τ 6·42 (s, 3H), 6·29 (s, 3H), 6·12 (s, 3H), 5·34 (s, 2H), 4·83 (s, 1H), 4·33 (s, 1H), 2·70 (m, 3H). MS m/e 304 (M^+) 273 (M-OMe), 272 (M-MeOH), 257, 245, 229, 213, 201. $C_{16}H_{16}O_6$ requires C, 63·15; H, 5·30; Found: C, 63·50; H, 5·39%.

(ii) Coumarin B ((**19**), 32 mg) R_f 0·2, prisms from EtOAc. M.p. 185–188°C. MS 290 (M^+) 275, 273, 272, 259, 246, 243, 229. $FeCl_3$ test negative. UV λ_{max}^{MeOH} 305 (sh), 284, 274, 230, 215 nm. $NaHCO_3$ liberates CO_2. Treatment of **19** with CH_2N_2 gave **18** identified by its spectra and m.m.p.

(iii) Coumarin C (**20**) R_f 0·6 white crystals from EtOAc (94 mg). M.p. 178–180°C. UV λ_{max}^{MeOH} 305 (sh) (ε 5·000), 284 (ε 12·000), 273 (ε 13·000), 215 (ε 19·500) nm IR ($CHCl_3$) ν_{max} 1700 (s), 1600 (s) cm^{-1}. NMR ($CDCl_3$) τ 7·80 (s, 3H), 6·10 (s, 3H), 5·88 (s, 2H), 4·30 (s, 1H), 2·67 (m, 3H). MS m/e 232 (M^+) 190b, 175, 160, metastables at 161 (190–175) and 155·7 (232–190). ($C_{13}H_{12}O_4$ requires C, 67·23; H, 5·21; Found: C, 67·42; H, 5·38%).

Condensation of Coumarin C (**20**). Coumarin (**20**, 35 mg) was refluxed in MeOH (30 ml) containing NaOMe

(120 mg Na) for 1 hr, (N_2). The cooled solution was acidified to pH 0 with 2N HCl, most of the MeOH evaporated and the mixture extracted with $CHCl_3$. Evaporation of the $CHCl_3$ gave a white gum, one spot on TLC (silica-$CHCl_3$/2% EtOH) R_f 0·4 which crystallized on standing. Recrystallized from EtOAc. M.p. 164–170°C. Sublimation gave white crystals of **21** m.p. 169–170°C. UV λ_{max}^{MeOH} 362 (ε 9·050), 347 (ε 11·200), 321 (ε 10·100), 306 (ε 11·000), 291 (ε 18·100), 280 (sh) (ε 13·600), 247 (ε 18·300), 237 (ε 13·100), 212 (ε 24·300). IR ν_{max} 1710 (s), 1670 (s), 1630 (s), 1600 (s) cm.$^{-1}$ MS *m/e* 200 (M^+), 172 (M-CO), 144 (172-CO), 116 (144-CO), metastables at 148 (200–172), 120·5 (172–144), 92·92 (144–116). ($C_{12}H_8O_3$ requires C, 72·0; H, 4·0; Found: C, 71·8%; H, 4·14%).

Annulation of 4-*hydroxy*-6-*carbomethoxymethyl*-2-*pyrone* (**27**). The pyrone (1·2 g) and bis (2,4-dichlorophenyl)malonate (3·6 g) mole ratio (1:1·5 respectively, were intimately mixed and heated on an oil bath, maintained at 195°C, for 2 min. The dark red product was eluted through a silica column with C_6H_6, C_6H_6/$CHCl_3$ mixtures and $CHCl_3$. The first few fractions contained phenolic compounds. The fractions eluted in $CHCl_3$ were evaporated yielding a pale yellow crystalline product (**28**, 1·37 g, 83%). Recrystallization from EtOAc yielded colourless prisms. M.p. 158–160°C. UV λ_{max}^{MeOH} 326 (ε 7·500), 269 (ε 10,7 0), 215, (ε 13,600) nm. IR (KBr) ν_{max} 3200 (w), 1745, 1735, 1700, 1640, 1620 cm.$^{-1}$ NMR ($CDCl_3$), τ 6·30 (s, 2H), 6·18 (s, 3H), 4·35 (s, 1H), 3·42 (s, 1H), −0·42 (br, 1H). MS *m/e* 252 (M^+) 224, 182, 165, 69b. ($C_{11}H_8O_7$ requires C, 52·39; H, 3·20; Found: C, 52·26; H, 3·06%).

Methyl dipyrone ester (29, R = Me). The dipyrone ester (**28**, 100 mg) was dissolved in dioxane (20 ml). An excess of CH_2N_2 in MeOH/ether (1·1%) was added and the solution allowed to stand overnight. Evaporation of the solvent yielded off-white crystals (100 mg, 95%) of methoxydipyrone ester (**29**). M.p. 183–184°. UV λ_{max}^{MeOH} 323 (ε 7000), 266 (ε 9400), 210 (ε 18,800) nm. IR (KBr) ν_{max} 1755, 1745, 1740, 1650 cm^{-1}. NMR ($CDCl_3$) τ 6·35 (s, 2H), 6·18 (s, 3H), 6·03 (s, 3H), 4·40 (s, 1H), 3·57 (s, 1H). MS *m/e* 266 (M^+) 238, 208, 168b, 253, 140, metastable at *m/e* 213 (266–238).

The methoxydipyrone acid (29, R = H). Methoxydipyrone ester (**29**, R = CH_3, 40 mg) was refluxed in 0·1 M HCl (10 ml) for 1 hr. After cooling, the solution was extracted with EtOAc (3 × 25 ml), washed, dried over Na_2SO_4 and evaporated yielding pale yellow crystals of methoxydipyrone acid (**29**, R = H, 35 mg, 92%). M.p. 250°C. UV λ_{max}^{MeOH} 321 (ε 6000), 266 (ε 7200), 212 (ε 17,400). IR (KBr) ν_{max} 3100 to 2500 (several), 1720, 1670, 1650, 1640 cm^{-1}. NMR (CF_3CO_2H) τ 6·07 (s, 2H), 5·85 (s, 3H), 3·93 (s, 1H), 3·17 (s, 1H). ($C_{11}H_8O_7$ requires C, 52·39; H, 3·20; Found: C, 51·56; H, 3·13%).

Preparation of sym dipyrone enol acetate (30). Methoxydipyrone acid (**29**, R = H, 200 mg) and anhyd NaOAc (500 mg) were stirred in acetic anhydride (30 ml) for 4 hr. Distilled H_2O (50 ml) was added and the mixture stirred for 2 hr to decompose the acetic anhydride. The mixture was extracted with $CHCl_3$ (4 × 150 ml), washed with H_2O, dried over Na_2SO_4 and evaporated yielding a brown residue. This was recrystallized from acetone/H_2O (3:1) yielding yellow microcrystalline product (**30**, 97 mg, 51%). M.p. 235–238°C. UV λ_{max}^{MeOH} 338 (ε 5500), 266 (ε 7100), 225 (ε 6500) nm. Addition of base gives $\lambda_{max}^{MeOH/OH^-}$ 648 nm which gradually diminishes. IR (KBr) ν_{max} 1755, 1720, 1660, 1640, 1600 cm^{-1}. NMR (CF_3CO_3H) τ 7·47 (s, 3H), 6·12 (s, 2H), 5·85 (s, 6H), 3·95 (s, 2H), 3·70 (s, 1H), 3·28, 3·22 (2H). MS (computerized MS 902) *m/e* 442 (M-42, common with enol acetates), 358, 235, 208b, 193. Metastable at *m/e* 125 (442–235).

Reaction of methoxydipyrone enol acetate (**30**) *with* 1 M NaOH aq. (i) Methoxydipyrone enol acetate (**30**, 240 mg) was stirred in 1 M NaOH aq (15 ml) at room temperature and the UV spectrum of the solution taken at intervals. After 30 min the peak at λ_{max} 648 had diminished to less than 1% of its original intensity. The mixture was cooled in an ice bath and acidified to pH 2 with 2 M HCl precipitating a light brown residue. This was filtered, washed with H_2O and dried to give an amorphous powder (120 mg). UV λ_{max}^{MeOH} 438, 302, 270, 213 nm, a drop of acid gave λ_{max} 320, 265, 211 nm. NMR (TFA) τ 5·87, 3·98, 3·23 (ratio of peaks approximately 8:2:2). IR (KBr) ν_{max} 3440 broad, 3100, 3000–2800 several, 1730, 1640, 1590, 1540 cm^{-1}. MS (very low intensity, peaks at *m/e* 442 and 398).

(ii) The product from above (10 mg) was refluxed in 1 M NaOH (10 ml) for 30 min. The solution was cooled and acidified to pH 2 with 2 M HCl. The mixture was evaporated to dryness, extracted with acetone and the acetone evaporated yielding a brown residue (3 mg). UV λ_{max}^{MeOH} 305, 225 (sh), 211 nm. MS highest peak at *m/e* 168 (spectrum very weak).

REFERENCES

[1] T. Money, I. H. Qureshi, G. R. B. Webster and A. I. Scott, *J. Amer. Chem. Soc.* **87**, 3004 (1965)
[2] T. Money, J. L. Douglas and A. I. Scott, *Ibid.* **88**, 624 (1966)
[3] F. Comer, T. Money and A. I. Scott, *Chem. Commun.* 231 (1967)

[4] T. Money, F. Comer, G. R. B. Webster, I. G. Wright and A. I. Scott, *Tetrahedron* **23**, 3435 (1967)
[5] D. G. Pike, J. J. Ryan and A. I. Scott, *Chem. Commun.* 629 (1968)
[6] H. Guilford, A. I. Scott, D. Skingle and M. Yalpani, *Ibid.* 1127 (1968)
[7] A. I. Scott, *Chimia* **22** (1968)
[8] Paper VIII. A. I. Scott. H. Guilford. J. J. Ryan and D. Skingle. *Tetrahedron* **27**. 3025 (1971)
[9] Paper IX. A. I. Scott. H. Guilford and D. Skingle. *Tetrahedron* **27**. 3039 (1971)
[10] T. M. Harris, M. P. Wachter and G. A. Wiseman, *Chem. Commun.* 177 (1969)
[11] A. J. Birch, D. W. Cameron and R. W. Rickards, *J. Chem. Soc.* 4395 (1960)
[12] H. Stetter and S. Vestner, *Ber. Dtsch. Chem. Ges.* **97**, 169 (1964)
[13] G. Bram, *Tetrahedron Letters* 4069 (1967)
[14] T. M. Harris and R. Carney, *J. Amer. Chem. Soc.* **89**, 6734 (1967)
[15] T. M. Harris and R. Carney, *Ibid.* **88**, 2053 (1966)
[16] T. M. Harris and R. Carney, *Ibid.* **88**, 5686 (1966)
[17] T. T. Howarth, G. P. Murphy and T. M. Harris, *Ibid.* **91**, 517 (1969)
[18] T. M. Harris and T. T. Howarth, *Chem. Commun.* 1253 (1968)
[19] J. L. Douglas and T. Money, *Can. J. Chem.* **46**, 695 (1968)
[20] G. D. Shah and R. C. Shah, *J. Sci. Ind. Res.* **15B**, 630 (1956)
[21] D. G. Pike, *D. Phil.* Thesis, University of Sussex, 1970; see also C. T. Bedford, J. L. Douglas, B. E. McCarry and T. Money, *Chem. Commun.* 1091 (1968)
[22] H. J. Hagemeyer and D. C. Hull, *Ind. Eng. Chem.* **41**, 2920 (1949)
[23] R. Bentley and P. M. Zwitkowitz, *J. Amer. Chem. Soc.* **89**, 676, 681 (1967)
[24] M. Brenner and W. Huber, *Helv. Chim. Acta* **36**, 1109 (1953)