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STUDIES IN RELATION TO BIOSYNTHESIS—XXI*

ROSENONOLACTONE AND GIBBERELLIC ACID

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Abstract—The biosyntheses of rosenonolactone in *Trichothecium roseum* and of gibberellic acid in *Gibberella fujikuroi* have been studied by feeding experiments with $CH_3{}^{14}CO_2Na$ and (2-^{14}C)-mevalonic lactone. Preliminary accounts[1-3] of this work have been published.

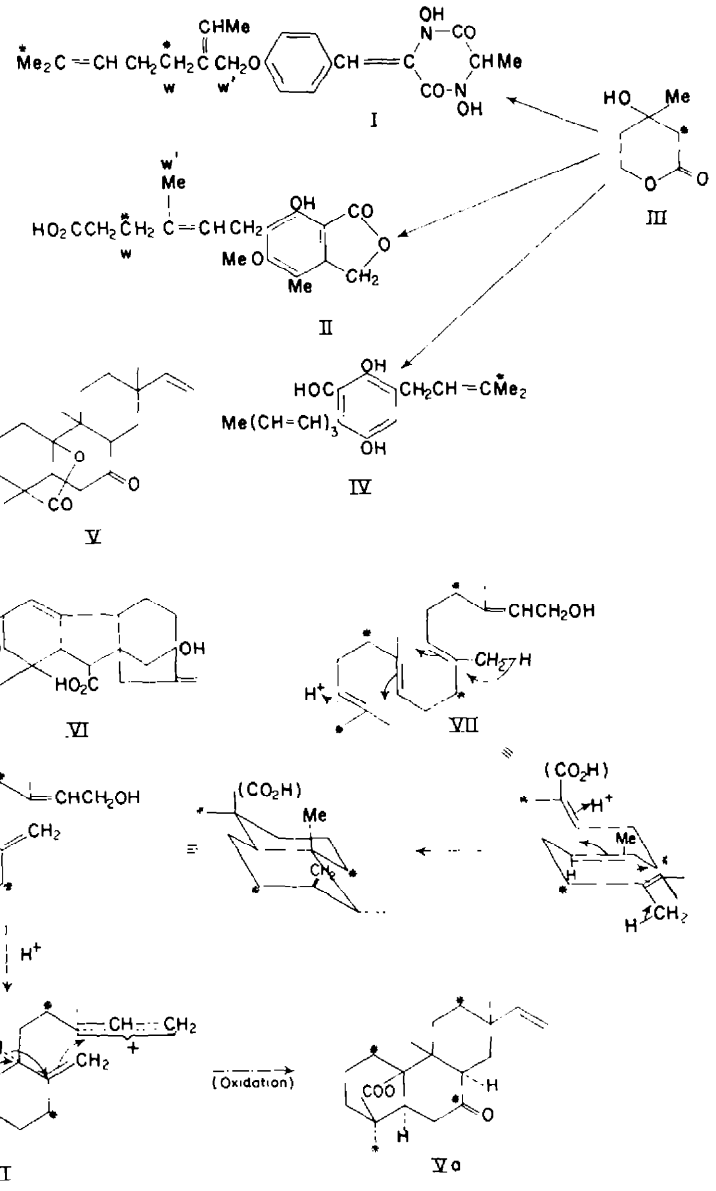
IN choosing moulds for experimental studies in the biosynthesis of terpenoid compounds we took account of the ease of culturing the organisms, the simplicity of techniques for incorporating substrates and the variety of terpenoid metabolites, including some with C_5, C_{10} or related units. The field therefore seemed to offer advantages over the classical squalene–lanosterol–cholesterol system hitherto investigated in liver or in yeast. Our first experiments[4] confirmed that the geranyl-type chain in mycelianamide (I), from *Penicillium griseofulvum*, and the oxidised geranyl chain in mycophenolic acid (II), from *P. brevi-compactum* are derived from mevalonic lactone (III) (C* = ^{14}C). More recently,[5] the simple isopentenyl side-chain of auroglaucin (IV) has also been shown to come from III. Evidence was also obtained in these organisms that mevalonic acid is derived from acetic acid.

In agreement with results obtained by other workers[6] for squalene and carotenoid biosynthesis, we observed that the *w*-carbon atoms (Klyne nomenclature[7]) of the isoprene units are derived specifically from the 2-position of mevalonic lactone. With (2-^{14}C)-mevalonic lactone as substrate, the label was confined to the main chains of I and II, there being no labelling on the *w'*-carbon atom. From this it is logical to inquire whether asymmetry of labelling persists in the terminal *gem*-dimethyl groups of a terpene chain. The difference between these groups is solely stereochemical, and by analogy with the normal *trans*-double bonds found in the chain of, for example, squalene, the methyl group *trans*- to the main chain should

* Part XX: A. J. Birch, O. C. Musgrave, R. W. Richards and H. Smith, *J. Chem. Soc.* In press.

[1] A. J. Birch, R. W. Richards and H. Smith, *Proc. Chem. Soc.* 192 (1958).
[2] A. J. Birch, A. Harris, R. W. Richards, H. Smith and W. B. Whalley, *Proc. Chem. Soc.* 223 (1958).
[3] A. J. Birch and H. Smith, *CIBA Symposium on Terpene and Sterol Biosynthesis* p. 245. John Churchill, London (1959).
[4] A. J. Birch, R. J. English, R. A. Massy-Westropp and H. Smith, *Proc. Chem. Soc.* 233 (1957); *Idem. J. Chem. Soc.* 369 (1958).
[5] A. J. Birch, J. Schofield and H. Smith, *Chem. & Ind.* 1321 (1958).
[6] J. W. Cornforth, R. H. Cornforth, G. Popjak and I. Youhotsky-Gore, *Biochem. J.* **66**, 10P (1957); **69**, 146 (1958); B. H. Amdur, H. Rilling, and K. Bloch, *J. Amer. Chem. Soc.* **79**, 2647 (1957); G. D. Braithwaite and J. W. Goodwin, *Biochem. J.* **67**, 13P (1957).
[7] W. Klyne, *Chem. & Ind.* 725 (1954).

contain all of the label. This situation is difficult to investigate experimentally in the readily available substances methylgeraniolene (obtained by reductive fission of



mycelianamide[4]) and squalene, biosynthesized from (2-^{14}C)-mevalonic lactone, because of the difficulty of distinguishing chemically between these two methyl groups. Asymmetry of labelling would be expected to persist in enzymatically cyclized products of such a chain, provided that the reactions are concerted and do not involve "free" cations. Questions covering distribution of label and the concerted mechanism of cyclization can be settled simultaneously by examining cyclic mould metabolites in which the relevant carbon atoms are chemically distinguishable.

Two suitable substances are known: rosenonolactone, structurally probably V,[8] and gibberellic acid (VI) the structure of which has been elucidated through the notable work of Grove, and his colleagues.[9]

Biosynthetically, rosenonolactone appears to be a diterpene which has suffered migration of a methyl group from the 12- to the 13-position. A route can be formulated as in the sequence (VII) → (VIII) → (Va) in line with current ideas on terpene cyclizations.[10]† No information is available as to the exact stages when the oxidations occur.

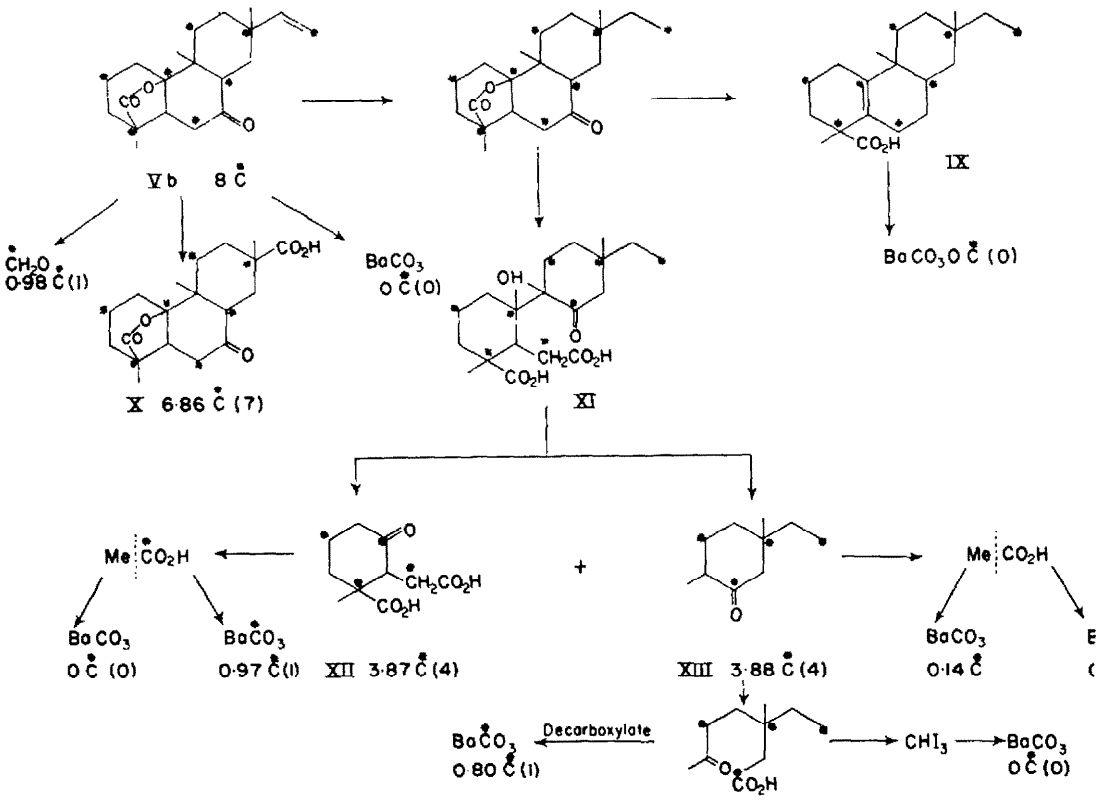
The metabolite was produced in cultures containing either $CH_3{}^{14}CO_2H$ or [2-^{14}C]-mevalonic lactone, the incorporation of radioactivity being 4 per cent in each case. The labelled material was degraded by methods chiefly developed in the earlier structure work.[8] The results are summarized in Schemes A and B on p. 244. The expected labelling patterns from $Me^{14}CO_2H$ and [2-^{14}C]-mevalonic lactone are Vb and Va respectively (C* = ^{14}C). The numbers of active carbon atoms shown for each degradation product are calculated from the radioactive assay data on the assumption that there are eight equally labelled carbon atoms in the rosenonolactone derived from $Me^{14}CO_2H$ and four when [2-^{14}C]-mevalonic lactone is the source. It will be seen that the results are in quantitative agreement with the expected distributions. Furthermore the derivation of the 1-Me group but not the 1-carbonyl group from the 2-position of mevalonic lactone confirms that the acyclic precursor must have the *gem*-dimethyl group unsymmetrically labelled and that the cyclization to the first two rings is a concerted one. Since the molar activity of ketone (XIII) is calculated on a formula $C_{10}H_{18}O$ the migration of the Me-group from the 12- to the 13-position is confirmed.

Work on gibberellic acid was pursued simultaneously. Inspection of the structure VI indicated a possible biosynthesis by a variant of the resin-acid process involving, besides oxidation, a number of additional steps for which there are biochemical or laboratory analogies. These include (a) oxidative loss of the methyl group attached to the 12-position; (b) formation of a phyllocladene-type bridged ring system from ring C and its substituents, and (c) ring contraction of ring B with extrusion of a carbon atom and formation of a cyclopentane carboxylic acid unit. Step (a) would be analogous to the loss of the angular group attached to the 14-position in the lanosterol–cholesterol conversion;[11] step (b) has a laboratory analogy in the acid-catalysed transformation of rimuene to phyllocladene[12] and, in view of the occurrence of diterpenes with oxygen substituents in the 9- or 10-positions or both (e.g. rosenonolactone, rosololactone[13] which occur together, and xanthopherol[14]) there is no difficulty in accepting the possibility that step (c) proceeds through a 9:10-dioxygenated derivative. A number of base-catalysed rearrangements for the change are available including: (i) a benzil-benzilic acid type of transformation of a 1:2-diketone leading to a 1-hydroxycyclopentane carboxylic acid; (ii) a Faworski-type rearrangement of

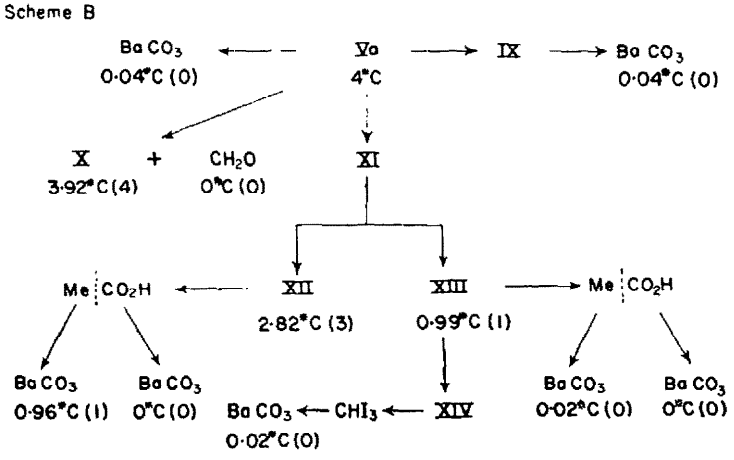
† See below for a discussion of the stereochemistry.

[8] A. Harris, A. Robertson and W. B. Whalley, *J. Chem. Soc.* 1799 (1958) and earlier papers.
[9] B. E. Cross, J. F. Grove, J. MacMillan, T. P. C. Mulholland and N. Sheppard, *Proc. Chem. Soc.* **221** (1958); T. P. C. Mulholland, *J. Chem. Soc.* 2693 (1958), and earlier papers.
[10] For a recent review see L. Ruzicka, *Perspectives in Organic Chemistry* (Edited by Todd) p. 265. Interscience, New York (1957).
[11] For a review see K. Bloch, *Vitamins and Hormones* Vol 15, p. 119. Academic Press, New York (1957).
[12] L. H. Briggs, B. F. Cain and J. K. Wilmshurst, *Chem. & Ind.* 599 (1958).
[13] A. Harris, A. Robertson and W. B. Whalley, *J. Chem. Soc.* 370 (1958).

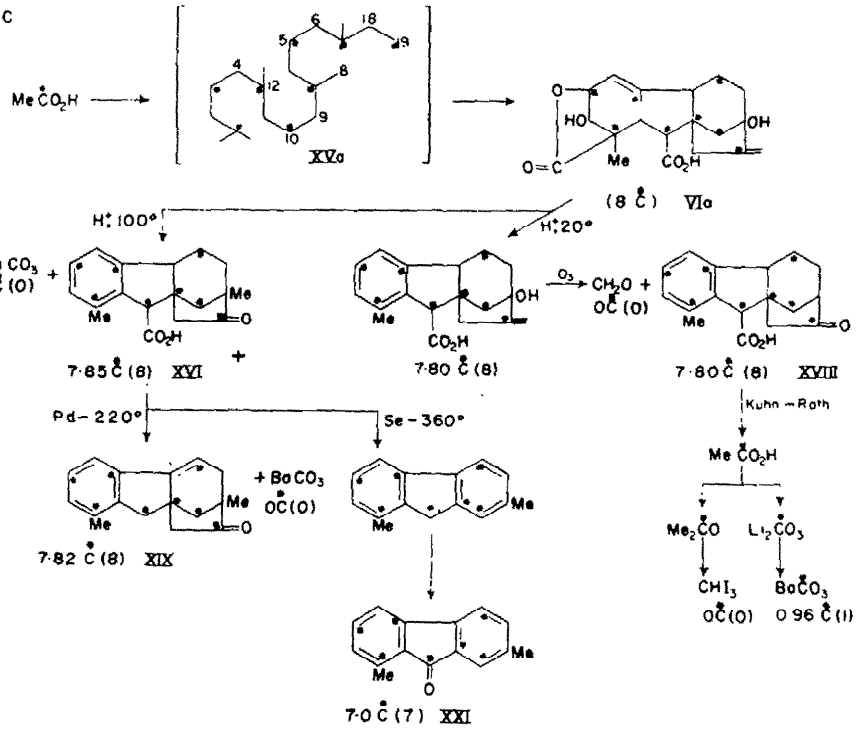
Scheme A

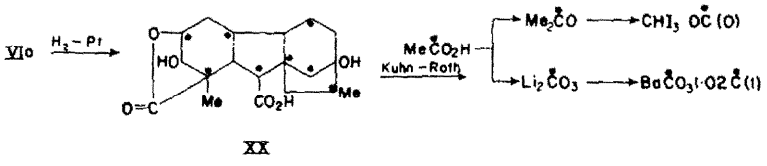


Scheme B

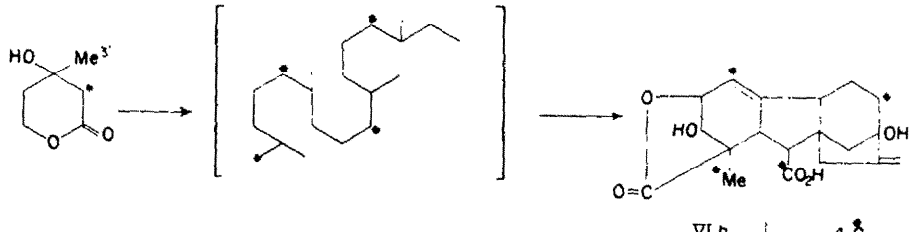


Scheme C





Scheme D



XVI + BaCO3
3·89 C*(4) 0·02 C*(0)

XVII → CH2O + XVIII
3·91 C*(4) 0·01 C*(0) 4·04 C*(4)

XXI
2·88 C*(3)

XIX + BaC*O3
2·92 C*(3) 1·01 C*(1)

Kuhn–Roth

Me*CO2H

Me2CO Li2CO3

CHI3 0·97 C*(1)

BaCO3 0C*(0)

an esterified 2-hydroxyketone leading directly to a cyclopentane carboxylic acid, and (iii) a rearrangement of a diesterified diequatorial cyclohexane-1:2-diol of a type observed to convert an 11-acetoxy (or tosyloxy)-12-tosyloxy (or acetoxy-) steroid into a C norsteroid,[15] from which a cyclopentane aldehyde would result.

We have been able to confirm the general accuracy of our view of the biosynthesis and to obtain information on the rearrangements involved by degrading gibberellic acid obtained from growth media containing either $CH_3{}^{14}CO_2H$ or (2-^{14}C)-mevalonic lactone. The incorporation of radioactivity was much higher with the latter substrate (2% compared with 0·2%). The degradation methods were essentially those used in the structure determination. The results are expressed as for rosenonolactone in Schemes C and D on p. 245.

The degradations are in quantitative agreement with the formation of gibberellic acid from 4 molecules of mevalonic lactone or 12 molecules of acetic acid. Reference to the predicted labelling patterns XVa and XVb in the diterpene presursor shows clearly that the lactone carbonyl carbon atom is derived specifically from the 3′-position of mevalonic lactone and that the carboxylic carbon of gibberellic acid arises specifically from the 9-position in the cyclic precursor.

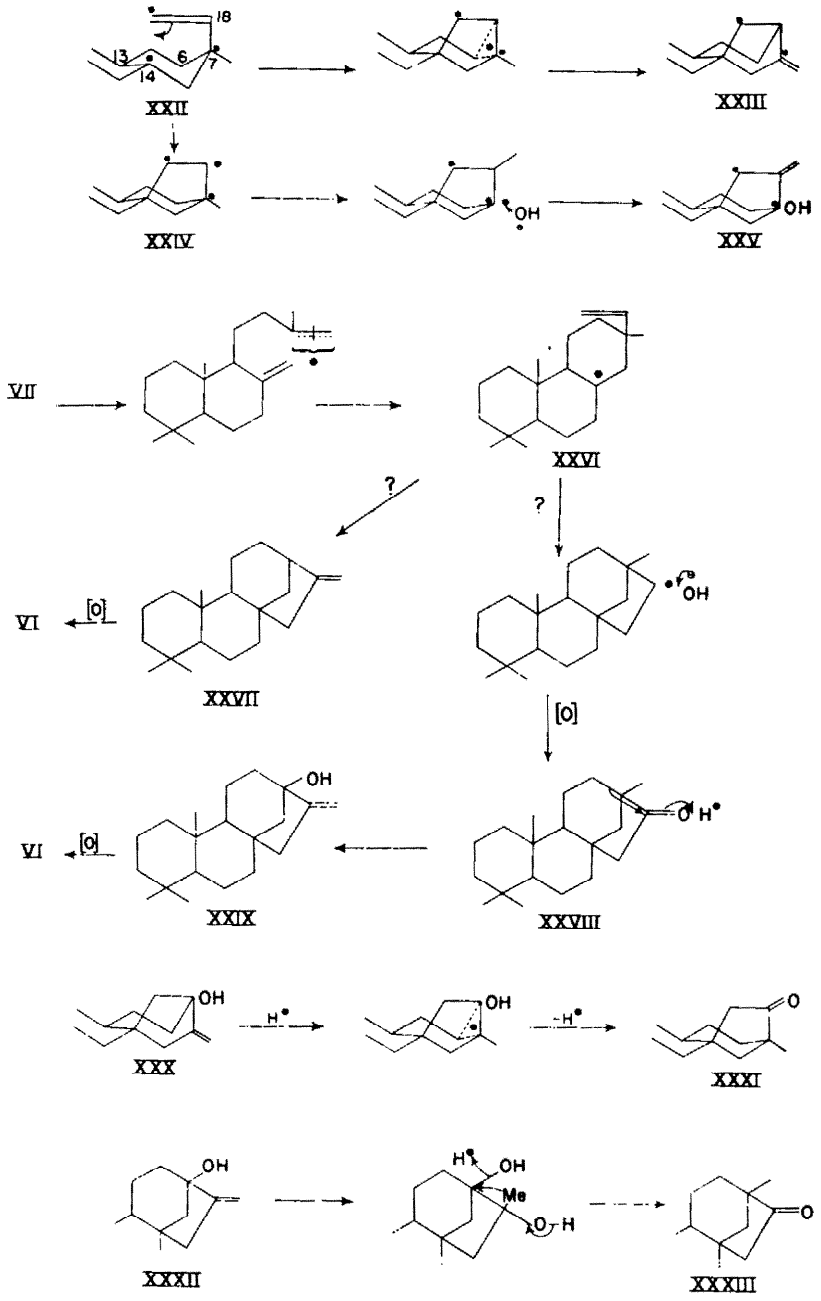
The degradations also illuminate the mechanism of formation of the bridged-ring system, a problem discussed already by Wenkert[16] on theoretical grounds in the case of the analogous phyllocladene system. A likely mechanism, as shown in the sequence (XXII) → (XXIII),* is attack of a carbonium ion at the 14-position on the 18:19-double bond of an axial vinyl group (quasi-axial in the non-protonated precursor). The overall result involves migration of $C_{(8)}$ from $C_{(7)}$ to $C_{(18)}$, i.e. the original Me group (becoming $=CH_2$ in the final product) remains attached to a labelled carbon atom if Me^*CO_2H is the precursor. In fact, the Kuhn–Roth oxidation on tetrahydrogibberellic acid confirms this attachment. An alternative, but theoretically much less likely route,[16] shown in XXIV → XXV involves migration of the Me to the $C_{(18)}$ (unlabelled carbon) and is ruled out by this evidence. In phyllocladene biosynthesis the $C_{(14)}$ cation could be produced by protonation of the 8:14-double bond in a rimuene-type precursor; or it could result as in gibberellic acid from a cationoid cyclization of the type shown in VII → XXVI → XXVII.

As with rosenonolactone, no attempt can be made at this stage to indicate the position of oxidation steps in the sequence. In order to explain the presence of the hydroxyl group at the bridge-head it is tempting to postulate an incompletely concerted reaction with intermediate production of a ketone of type XXVIII rearranging to XXIX. There is at present no evidence to distinguish such a scheme from the alternative involving the transformation XXII → XXIII followed by hydroxylation.

The reaction sequence XXX → XXXI is the "obvious" one[17] for the change gibberellic acid into gibberic acid (so far as this part of the molecule is concerned), the reaction being initiated by protonation of the $=CH_2$. Using gibberellic acid derived from $Me^{14}CO_2H$ this conversion would give gibberic acid in which both Me

* These and subsequent perspective diagrams are drawn for diterpene skeletons having the C_{10}-methyl group β, i.e. of the same stereochemical series as cholesterol.

[14] J. B. Bredenberg, *Acta Chem. Scand.* **11**, 927 (1957).
[15] R. F. Hirschmann, H. L. Slates, R. W. Walker and N. L. Wendler, *Chem. & Ind.* 901 (1954).
[16] E. Wenkert, *Chem. & Ind.* 282 (1955).

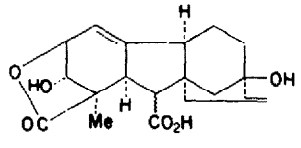


groups would remain attached to labelled carbon atoms. In fact Kuhn–Roth oxidation gave acetic acid with approximately one half of the expected activity. This result could indicate that the gibberellic–gibberic acid transformation involves migration of the methylene carbon atom (presumably as a methyl group) to an inactive carbon atom. This could possibly occur as a consequence of preliminary hydration of the

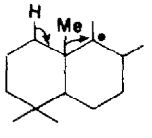
[17] B. E. Cross, J. F. Grove, J. MacMillan and T. P. C. Mulholland, *Chem. & Ind.* 954 (1956).

methylene double bond followed by a pinacol–pinacolone rearrangement XXXII → XXXIII, but this sequence, which implies an intermediate having a carbonium ion centre at the bridgehead position, appears mechanistically improbable. Elucidation of the stereochemical interrelationship between the two acids should settle this point.

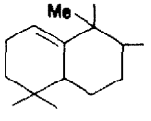
The above verification of the terpenoid character of rosenonolactone and gibberellic acid permits a number of reasonable speculations on the stereochemistry of each metabolite. Infra-red evidence indicates that the lactone carbonyl groups in both substances form part of an unstrained five-membered ring which accordingly must be attached diaxially to the ring system. These groups have been shown to be derived specifically from the 3'-position in mevalonic lactone thus providing circumstantial evidence that the initial diterpene cyclisation is stereochemically similar in both biosyntheses. Enzymic cyclisation of acyclic terpenes is known or believed to give the *trans-anti*-arrangement of the asymmetric centres at the 11-, 12- and 13-positions in diterpenes* (and in analogous positions in triterpenes) and it is reasonable to presume a similar stereochemistry in the precursors of rosenonolactone and gibberellic acid. In the first case the incorporation of an axial carbon substituent in the 1-position into the bridged lactone ring is most simply interpreted as occurring through a concerted reaction involving migration of the methyl group from the 12- to the 13-position as already shown above in VII and VIII. It would therefore be predicted that the stereochemistry of rosenonolactone is represented by Va or a diastereoisomeric structure, provided that later epimerisation at the 14-position (which would be permitted by the adjacent carbonyl group) does not occur. Evidence[18] confirming the stereochemical assignment Va was obtained later. In the case of gibberellic acid the rearrangement for formation of rings C and D which we have demonstrated above defines the stereochemical interrelationships at the 7-, 13- and 14-positions in the diterpene precursor and the stereo-chemistry which would be predicted depends on whether the methyl group attached to the 12-position is oxidized *in situ* or after a migration reaction. In the first case oxidative removal of the methyl group at the 12-position would not be expected to disturb the stereochemistry (cf. lanosterol oxidation) and, taking into account the fact that the hydroxyl in ring A is axial,



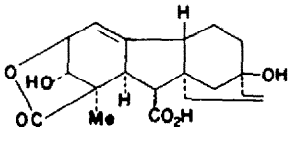
XXXIV



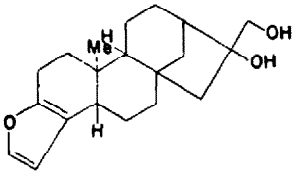
XXXV



XXXVI



XXXVII



XXXVIII

* But see E. Wenkert and J. W. Chamberlain, *J. Amer. Chem. Soc.* **81**, 688 (1959).

[18] B. Green, A. Harris, W. B. Whalley and H. Smith, *Chem. & Ind.* 1369 (1958).

gibberellic acid is defined as XXXIV.* The presence of the double bond in ring A suggests the additional possibility that the methyl group is removed from the 13-position after a migration of the type shown in XXXV and XXXVI. This would involve an inversion at this position and gibberellic acid would then be represented by XXXVII.* It has been suggested[19] that cafestol possesses the structure and absolute configuration XXXVIII (opposite stereochemical series to cholesterol). It is of interest that this structure is consistent with a biosynthesis paralleling the early stages of gibberellic acid synthesis demonstrated here.

EXPERIMENTAL

General directions are as for Part XIX—Relative molar activities (r.m.a.'s) (a) and (b) refer to labelled metabolites and corresponding degradation products derived from $Me^{14}CO_2Na$ and (2-^{14}C)-mevalonic lactone respectively. Theoretical numbers of labelled carbon atoms and r.m.a.'s refer to the appropriate postulated distributions of radioactivity. Degradation methods for rosenonolactone and gibberellic acid are these of the earlier structural work; only modifications are described in detail. M.p.s. were measured on the Kofler bloch and are uncorrected. Light petroleum means the fraction b.p. 40–60°.

(^{14}C) Rosenonolactone

(a) *Trichothecium roseum* Link was grown as previously described. After 4 weeks aqueous $Me^{14}CO_2Na$ (0·25 mc) was distributed between 4 flasks each containing 750 cc of medium. After a further 2 weeks $Me^{14}CO_2Na$ (0·25 mc) was added similarly and growth was allowed to proceed for a total of 8 weeks. The mycelium was harvested in the usual way to give, from benzene–light petroleum, rosololactone (0·25 g) m.p. 184–186°. (Found: r.m.a. × 10^{-4}, 261) and impure rosenonolactone (1·54 g), an aliquot of which was purified (Found: r.m.a. × 10^{-4}, 195). The remainder (1·0 g) was combined with pure unlabelled lactone (6 g) and labelled lactone obtained by carrier extraction of the previously removed fat fraction with pure unlabelled lactone (3 × 0·9 g). Recrystallization from ethanol gave rosenonolactone m.p. 212–214° (20 μc; 4%). (Found: r.m.a. × 10^{-4}, 25·4).

(b) (2-^{14}C) Mevalonic lactone (0·2 mc) was incorporated into the culture medium as for $Me^{14}CO_2Na$, giving rosenonolactone (7·9 μc; 4%). (Found: r.m.a. × 10^{-4}, 9·85).

Ozonolysis of (^{14}C) rosenonolactone

The lactone (350 mg) was ozonized in acetic acid (40 cc) at room temp. One seventh of the solution was stirred with zinc dust (150 mg) and water (0·5 cc) for 3 hr. Steam distillation gave formaldehyde obtained as the 2,4-dinitrophenylhydrazone m.p. (from aqueous ethanol) 167–169°. (Found: r.m.a. × 10^{-4}, (a) 3·13; (b) 0.1 C requires (a) 3·18; (b) 2·46). 30% Hydrogen peroxide (3 cc) was added to the remaining ozonolysis solution. After 20 hr excess reagent was decomposed catalytically with palladized charcoal, water was added and the product was collected with ether. The portion which was soluble in aqueous sodium carbonate gave, after recrystallization from aqueous ethanol the acid (X) (172 mg) m.p. 258–260°. (Found: r.m.a. × 10^{-4}, (a) 21·8; (b) 9·65. 7*C, 4*C require respectively (a) 22·3; (b) 9·85).

Decarboxylation of (^{14}C) rosenonolactone

(i) The lactone (64 mg) and naphthalene-2-sulphonic acid (20 mg) were maintained at 160° for 10 min (vigorous gas evolution) in a stream of nitrogen. The effluent carbon dioxide was collected as barium carbonate (37 mg, 0·92 mole). (Found: r.m.a. × 10^{-4}, (a) 0; (b) 0·11. 1*C requires (a) 3·18; (b) 2·46). The neutral fraction of the residual oil had a single ν max at 1660 cm^{-1} in the C=O stretching region.

(ii) Isoros-11,12-en-16-oic acid (100 mg) was heated at 250° for 1 hr in a stream of nitrogen. The carbon dioxide was collected as barium carbonate (46 mg, 0·70 mole). (Found: r.m.a. × 10^{-4},

* Or the enantiomer.

[19] M. Cais, C. Djerassi and L. A. Mitscher, *J. Amer. Chem. Soc.* **80**, 247 (1958).

(a) 0; (b) 0·10. 1*C requires respectively (a) 3·18; (b) 2·46). The neutral residue had no carbonyl or hydroxyl absorption in the infra-red.

(^{14}C)-Rosoic acid

Dihydrorosenonolactone (200 mg) was refluxed in 0·5 N ethanolic sodium hydroxide (3 cc) for 30 min. The ethanol was removed under reduced pressure, the volume was made up to 20 cc with water and potassium permanganate (200 mg), sodium carbonate (200 mg) was added portionwise with stirring over 4 hr. After a further 2 hr the solution was acidified and decolorized with sodium sulphite and the product was collected with ether.. The acidic fraction gave, after recrystallization from ethyl acetate–light petroleum, rosoic acid (XI) (170 mg) m.p. 228–230°.

Scission of (^{14}C) rosoic acid

Rosoic acid on retroaldolization gave the ketone (XIII) as an oil assayed as the semicarbazone m.p. (from ethanol) 176–178°. (Found: r.m.a. × 10^{-4}, (a) 12·3; (b) 2·42. 4*C, 1*C require respectively (a) 12·7; (b) 2·46), and the acid (XII) m.p. (from ethyl acetate) 205°. (Found: r.m.a. × 10^{-4}, (a) 12·3; (b) 6·92. 4*C, 3*C require respectively (a) 12·7; (b) 7·38). Kuhn–Roth oxidation of the above semi-carbazone gave acetic acid (1·50, 1·48 moles in replicate experiments) obtained as the lithium salt which was degraded further to $BaCO_3$ (Me). (Found: r.m.a. × 10^{-4}, (a) 0·42; (b) 0·05. 1*C requires (a) 3·18; (b) 2·46) and $BaCO_3$ (CO_2H). (Found: r.m.a. × 10^{-4} (a) 0·38; (b) 0). Kuhn–Roth oxidation of the acid (XIII) gave lithium acetate (0·40, 0·41 mole) further degraded to $BaCO_3$ (Me). (Found: r.m.a. × 10^{-4}, (a) 0; (b) 2·36. 1*C requres (a) 3·18; (b) 2·46) and $BaCO_3$ (CO_2H) (Found: r m.a. × 10^{-4}, (a) 3·080 (b) 0).

Ozonolysis of the ketone (XIII) gave the oily ketoacid (XIV) which with aqueous sodium hypoiodite gave iodoform converted in the usual manner to $BaCO_3$. (Found: r.m.a. × 10^{-4}, (a) 0; (b) 0·04).

Concentrated sulphuric acid (1 cc) was added to the above ketoacid (34 mg) and sodium azide (75 mg) in benzene (3 cc) in a stream of nitrogen. The effluent carbon dioxide was collected as $BaCO_3$ (31 mg., 0·85 mole). (Found: r.m.a. × 10^{-4}, (a) 2·54; 1*C requires (a) 3·18).

(^{14}C) Gibberellic acid

(a) *Gibberella fujikuroi* was cultured in a medium (5 l.) containing glucose and ammonium nitrate, and a solution of $Me^{14}CO_2Na$ (0·5 mc) was added when the glucose was almost exhausted. The crude labelled giberellic acid (0·54 g) was combined with pure acid (6 g) and recrystallized from ethyl acetate–methanol to give (^{14}C) gibberellic acid m.p. 233–235° (0·85 μc; 0·17%. (Found: r.m.a. × 10^{-3}, 58·8).

(b) (2-^{14}C) Mevalonic lactone (0·2 mc) was incorporated as for $Me^{14}CO_2Na$ giving (^{14}C) gibberellic acid (4·8 μc; 2·4%). (Found: r.m.a. × 10^{-3}, 109).

Degradations of (^{14}C) gibberellic acid

(i) Gibberellic acid (300 mg) was converted to gibberic acid (XVI) (230 mg) m.p. 157–159° (Found: r.m.a. × 10^{-3}, (a) 57·8; (b) 106. 8*C, 4*C require respectively (a) 58·8; (b) 109) and barium carbonate (146 mg, 0·85 mole). (Found: r.m.a. × 10^{-3}, (a) 0; (b) 0·53. 1*C requires respectively (a) 7·35; (b) 27·3). Kuhn–Roth oxidation of gibberic acid gave acetic acid (0·62, 0·64 mole), degraded further to $BaCO_3$ (Me). (Found: r.m.a. × 10^{-3}, (a) 0) and $BaCO^3$ (CO_2H). (Found: r.m.a. × 10^{-3}; (a) 3·75. 1*C requires (a) 7·35), and also obtained as *p*-bromophenacylacetate m.p. 86°. (Found: r.m.a. × 10^{-3}, (a) 4·47. 1*C requires (a) 7·35).

(ii) Gibberic acid (220 mg) on dehydrogenation with carbon dixode-free palladium charcoal was converted to gibberone (100 mg) (XIX) m.p. 120–123°. (Found: r.m.a. × 10^{-3}, (a) 57·5; (b) 79·6. 8*C, 3*C require respectively (a) 58·8; (b) 82·0), and barium carbonate (107 mg, 0·70 mole). (Found: r.m.a. × 10^{-3}, (a) 0; (b) 27·5. 1*C requires respectively (a) 7·35; (b) 27·3).

(iii) Gibberellic acid (2·2 g) was converted to allogibberic acid (XVII) (1·14 g) m.p. 200–203°. (Found: r.m.a. × 10^{-3}; (a) 57·3; (b) 107. 8*C, 4*C require respectively (a) 58·8; (b) 109). allo-Gibberic acid (120 mg) was ozonized at room temp in acetic acid (5 cc). Water (2 cc) was added and the solution was stirred with zinc dust (400 mg) for 2 hr. Steam distillation gave formaldehyde, obtained as the 2,4-dinitrophenylhydrazone (60 mg; 0·66 mole) m.p. 167–168°. (Found: r.m.a. × 10^{-3},

(a) 0; (b) 0·29. 1*C requires (a) 7·35; (b) 27·3). The product obtained by extraction of the residue with ether was recrystallized from ethyl acetate–light petroleum to give the ketol (XVIII) (70 mg) m.p. 255–258° (decomp). (Found: r.m.a. $\times 10^{-3}$, (a) 59·9; (b) 110. 8*C, 4*C require respectively (a) 58·8; (b) 109),

Kuhn–Roth oxidation of the ketol (XVIII) gave acetic acid (0·43, 0·50 mole) degraded to $BaCO_3$ (Me). (Found: r.m.a. $\times 10^{-3}$, (a) 0; (b) 26·4. 1*C requires respectively (a) 7·35; (b) 27·3) and $BaCO_3$ (CO_2H). (Found: r.m.a. $\times 10^{-3}$, (a) 7·05; (b) 0).

(iv) Impure gibberic acid (0·67 g) was dehydrogenated with selenium. The impure gibberene (1,7-dimethylfluorene) (0·27 g) in acetone (20 cc) was oxidized with potassium permanganate (900 mg) for 5 hr to yield gibberenone (1,7-dimethylfluorenone) (XXI) (180 mg) m.p. 75–77°. (Found: r.m.a. $\times 10^{-3}$, (a) 51·5; (b) 78·6. 7*C, 3*C require respectively (a) 51·5 (b) 81·9).

(v) Sodium gibberellate (from the acid, 303 mg) in water (5 cc) was shaken with Adam's catalyst (from PtO_2, 80 mg) under hydrogen. Uptake of hydrogen (2·2 moles) was complete in 20 min. Evaporation gave the sodium salt of the acid (XX) which was oxidized by the Kuhn–Roth procedure to acetic acid (1·04 mole), further degradation of which gave $BaCO_3$ (Me) (Found: r.m.a. $\times 10^{-3}$; (a) 0) and $BaCO_3$ (CO_2H). (Found: r.m.a. $\times 10^{-3}$, (a) 7·50. 1*C requires (a) 7·35).

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