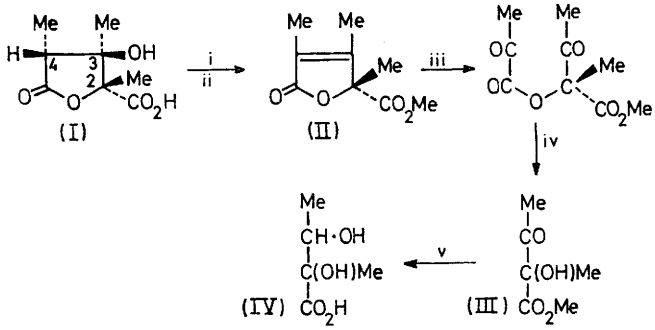
# Pyrrolizidine Alkaloids. The Absolute Configuration at C-2 in Monocrotalic Acid

By D. J. Robins and D. H. G. Crout,* Department of Chemistry, University of Exeter, Stocker Road, Exeter, Devon

Evidence is presented which firmly establishes the *R*-configuration at C-2 in monocrotalic acid. In addition, (−)-methyl 2-hydroxy-2-methyl-3-oxobutanoate (methyl acetolactate) is shown to have the *R*-configuration.



THE (2*R*,3*R*,4*R*)-configuration has recently been assigned to monocrotalic acid (I), the necic acid component of the pyrrolizidine ester alkaloid monocrotaline.[1,2] The proposed relative configuration at centres C-2 and C-3 conflicts with the previous assignment (2*R*,3*S* or 2*S*,3*R*) of Adams and his co-workers,[3] who based their assignment on the reasonable assumption that in their synthesis of monocrotalic acid, hydroxylation of *cis*-1,2,3-trimethylpropene-1,3-dicarboxylic acid with pertungstic acid took place in a stereospecifically *trans* manner. Possible explanations for the anomalous stereochemical course of this reaction have been put forward.[1] However, the existence of this discrepancy made it desirable to obtain further evidence for the configuration at C-2 in monocrotalic acid (I), since although both chemical[1] and spectroscopic (n.m.r.[1] and o.r.d.[2]) evidence favoured the *R*-configuration, the arguments adduced in support of this assignment were in each case indirect.



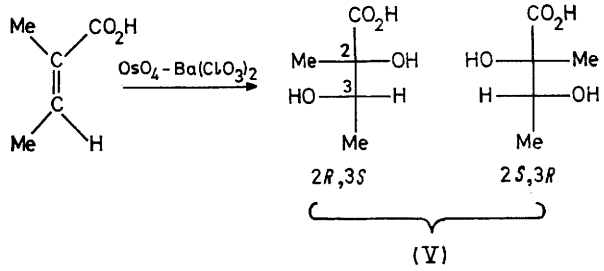

SCHEME 1

Reagents: i, $CH_2N_2$; ii, $POCl_3$–pyridine; iii, $O_3$; iv, $NaHCO_3$; v, $NaBH_4$; $Ba(OH)_2$

Methyl anhydromonocrotalate [(II), Scheme 1] was ozonised and the product was selectively hydrolysed to methyl 2-hydroxy-2-methyl-3-oxobutanoate (methyl acetolactate) (III) $[\alpha]_D^{24}$ −9·9°. Reduction of the ester (III) with sodium borohydride followed by hydrolysis gave a mixture of diastereoisomeric 2,3-dihydroxy-2-methylbutanoic acids (IV) with $R_F$ value identical with that of authentic *threo*-2,3-dihydroxy-2-methylbutanoic acid (V). (Separation of the diasteroisomers was not observed in the chromatographic systems employed.)

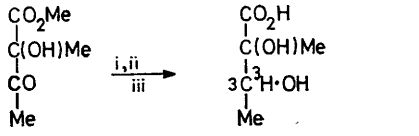
[1] D. J. Robins and D. H. G. Crout, *J. Chem. Soc.* (*C*), 1969, 1386.
[2] O. Červinka, L. Hub, A. Klásek, and F. Šantavý, *Chem. Comm.*, 1968, 261.
[3] R. Adams, B. L. Van Duuren, and B. H. Braun, *J. Amer. Chem. Soc.*, 1952, **74**, 5608.

The absolute configurations of the (+) and (−)-*threo*-acids (V) have been established by chemical correlation through to primary standards.[4] Correlation with the product from the reduction of (−)-methyl acetolactate was established as follows.




SCHEME 2

The racemic acid obtained by *cis*-hydroxylation of tiglic acid (Scheme 2) was resolved with brucine[5] to give the salt of the (2*R*,3*S*)-acid, m.p. 239—240°, $[\alpha]_D^{21}$ −21°, and the salt of the (2*S*,3*R*)-acid, m.p. 229—230°, $[\alpha]_D^{21}$ −29°. Reduction of (−)methyl acetolactate (from methyl monocrotalate; Scheme 1) with sodium borotritiide, followed by hydrolysis, gave a diastereoisomeric mixture consisting either of the (2*R*,3*R*)- and (2*R*,3*S*)-acids (IV) or of the (2*S*,3*S*)- and (2*S*,3*R*)-acids, labelled with tritium at C-3 (Scheme 3). The product




*cf.* Scheme 2

2*R*,3*R* + 2*R*,3*S*
or
2*S*,3*R* + 2*S*,3*S*

SCHEME 3

Reagents: i, $NaB^3H_4$; ii, $Ba(OH)_2$; iii, $H^+$

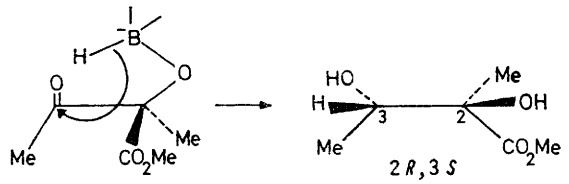
was shown to have a radiochemical purity of >99% (as the diastereoisomeric mixture) by paper chromatography with liquid scintillation scanning.

Aliquot portions of the labelled acid mixture were co-crystallised with the brucine salts of the authentic (2*R*,3*S*)- and (2*S*,3*R*)-acids, and the product salts were recrystallised to constant activity. The activity of the (2*S*,3*R*)-salt rapidly fell to 0·01% of its original value.

[4] W. Christensen and A. Kjaer, *Proc. Chem. Soc.*, 1962, 307.
[5] G. S. Myers, P. Morozovitch, W. L. Glen, R. Barber, G. Papineau-Couture, and G. A. Grant, *J. Amer. Chem. Soc.*, 1955, **77**, 3348.

However, the activity of the (2*R*,3*S*)-salt on repeated crystallisation reached a constant value corresponding to the entrainment of 32 ± 2% of the original activity. The labelled mixture of acids derived from (—)-methyl acetolactate thus consisted of 32% of the (2*R*,3*S*)-isomer and 68% of the (2*R*,3*R*)-isomer, showing that monocrotalic acid has the 2*R*-configuration, in confirmation of previous suggestions.[1,2]

Cram has shown that in nucleophilic addition to the carbonyl group of dissymetric ketones which bear hydroxy- or amino-functions on the α-carbon atom, the diastereoisomer formed in greatest amount is predicted by the so-called 'rigid model' of the transition state. This model assumes that the disposition of the substituents attached to the α-carbon, relative to the carbonyl function, is governed by bonding between the α-hydroxy- or amino-function and the attacking reagent species (*cf.* Scheme 4).[6] Attack on the carbonyl




SCHEME 4

function takes place preferentially from the side of the smallest of the two remaining α-substituents. If conformational energies are taken as a measure of effective steric size, then Me > $CO_2Me$ [7] and the (2*R*,3*S*)-isomer would be expected to preponderate in the mixture formed by borohydride reduction of methyl acetolactate (Scheme 4). Since the (2*R*,3*R*)-isomer was found to preponderate, it is concluded either that the 'rigid model' variation of Cram's rule of asymmetric induction fails for this particular compound, or that conformational energies do not provide a reliable indication of the relative effectiveness of Me and $CO_2Me$ in shielding the carbonyl group from hydride ion attack.

The present work establishes the absolute configuration of the laevorotatory methyl ester of acetolactic acid, which is an intermediate in the biosynthesis of valine and leucine.[8] Although the semicarbazone of (±)-methyl acetolactate (III) was readily obtained, the derivative of the (—)-isomer did not crystallise. However, the 2,4-dinitrophenylhydrazones of both (±)- and (—)-methyl acetolactate were successfully prepared. The c.d. curve of the derivative of the (—)-ester, kindly determined by Dr. P. M. Scopes and Professor W. Klyne, showed extrema at 212 (Δε +4·61), 233 (Δε —3·18) and 282 (Δε +1·42) nm. The low light transmission of solutions of the derivative precluded determination of the o.r.d. curve.

## EXPERIMENTAL

All m.p.s are corrected. I.r. spectra were determined with a Hilger H900 Infrascan spectrometer. N.m.r. spectra (60 MHz) were determined with a Perkin-Elmer R60 spectrometer. Optical rotations were measured with a Bellingham and Stanley Pepol 60 polarimeter (Unicam SP 500 source). Radioactivity measurements were made with a Packard Tri-Carb Series 2000 liquid scintillation counter. All activities were determined by use of NE 220 scintillation liquid (Nuclear Enterprises Ltd.). For paper chromatography of carboxylic acids on Whatman no. 1 paper, the systems (a) butanol–toluene–acetic acid–water (2 : 2 : 1 : 5 v/v) and (b) ethanol–conc. ammonia–water (80 : 5 : 10 v/v) were employed. Dihydroxy-acids were detected with a periodate–starch spray reagent.[9] Alumina for chromatography (B.D.H.) was washed twice with 50% nitric acid, followed by water until neutral and then methanol, and finally activated at 110° for 16 hr.

*Methyl Anhydromonocrotalate* (II).—Methyl monocrotalate (I) (3·8 g.) in pyridine (30 ml.) containing phosphoryl chloride (14·4 g.), was heated under reflux for 2 hr. The reaction mixture was cooled, treated with water (15 ml.), acidified (Congo Red) with conc. hydrochloric acid, and extracted with ether (4 × 500 ml.). The extract was dried ($MgSO_4$) and evaporated to give methyl anhydromonocrotalate (yellow oil) (2·6 g.), $\nu_{max.}$ (film) 1775 (lactone CO), 1750 (ester CO), and 1690 (C=C) cm.$^{-1}$, τ ($CDCl_3$) 6·24 (s,MeO), 8·01 and 8·17 (each s,MeC=), and 8·36 (s,Me).

(—)-*Methyl 2-Hydroxy-2-methyl-3-oxobutanoate* [(—)-*Methyl Acetolactate*] (III).—Methyl anhydromonocrotalate (II) (1 g.) in dry ethyl acetate (20 ml.) was ozonised for 6 hr. at 0°. Excess of ozone was removed ($N_2$) and the ozonide was decomposed by stirring with water (20 ml.) containing manganese dioxide (1·5 g.). The organic layer was separated and the aqueous layer was washed with ethyl acetate (2 × 20 ml.). The organic solutions were combined, the solvent was evaporated off, and the residual yellow oil was treated with 0·1M-sodium hydrogen carbonate (150 ml.) for 45 min. The solution was extracted continuously with ether for 20 hr., the extract was dried ($MgSO_4$), and the ether was removed to leave (—)-methyl acetolactate (III) (yellow oil) (400 mg.), $[\alpha]_D^{24}$ —9·9° (*c* 3·05 in EtOH), $\nu_{max.}$ (film) 3480 (OH) and 1750 and 1728 (CO) cm.$^{-1}$, τ ($CDCl_3$) 5·8 (s,OH), 6·18 (s,MeO), 7·72 (s,Me), and 8·39 (s,Me). The i.r. spectrum was identical with that of an authentic sample prepared according to the published procedure for preparation of the ethyl ester.[10] The 2,4-*dinitrophenylhydrazone*, purified by chromatography on alumina, crystallised (ethanol) as orange needles, m.p. 173·5—174°. The i.r. spectrum (KBr) was identical with that of the corresponding racemic derivative, m.p. 171—172° (Found: C, 44·2; H, 4·5; N, 16·95. $C_{12}H_{14}N_4O_7$ requires C, 44·15; H, 4·3; N, 17·15%). The *semicarbazone* of (±)-methyl acetolactate, after two recrystallisations (ethanol), had m.p. 147·5—148° (Found: C, 41·45; H, 6·4; N, 20·5. $C_7H_{13}N_3O_4$ requires C, 41·4; H, 6·45; N, 20·65%).

threo-2,3-*Dihydroxy-2-methylbutanoic Acid* (V).—A solution of tiglic acid (40 g.) in water (3·25 l.), containing

[6] D. J. Cram and K. R. Kopecky, *J. Amer. Chem. Soc.*, 1959, **81**, 2748.

[7] J. A. Hirsch, 'Topics in Stereochemistry,' vol. I, eds. N. L. Allinger and E. L. Eliel, Interscience, New York, London, and Sydney, 1967, p. 199.

[8] A. Meister, 'Biochemistry of the Amino-Acids,' Academic Press, New York and London, 1965, p. 730 *et seq.*

[9] R. L. Metzenberg and H. K. Mitchell, *J. Amer. Chem. Soc.*, 1954, **76**, 4187.

[10] L. O. Krampitz, *Arch. Biochem.*, 1948, **17**, 81.

osmium tetroxide (200 mg.) and barium chlorate monohydrate (32 g.), was stirred for 3 days. The solution was brought to pH 4 with 2M-hydrochloric acid (2 ml.) and extracted with benzene (3 × 600 ml.). The residual aqueous solution was concentrated at 40° to 100 ml., saturated with sodium chloride, and extracted continuously with ether for 2 days. The ethereal extract was dried ($MgSO_4$) and evaporated, and the residual orange oil (35 g.) gave the dihydroxy-acid (V) (14·5 g.), m.p. 86—87° (from ether) (lit.,[5] 87·5—88·5°), $\nu_{max.}$ (KBr) 3460 and 3340 (OH), and 1730 (CO) cm.$^{-1}$, $\tau$ (in $D_2O$ with sodium 3-trimethylsilylpropanesulphonate as internal standard) 5·99 (1H, q, *J* 7 Hz), 8·68 (s,Me), and 8·76 (d, *J* 7 Hz, *Me*CH), $R_F$ [system (a)] 0·55, [system (b)] 0·77.

2,3-*Dihydroxy*-2-*methyl*[3-$^3$H]*butanoic Acid*.—(−)-Methyl acetolactate (10 mg.) in ethanol (1 ml.) was added to sodium borotritiide (10 mCi; 110 mCi mmole$^{-1}$). The solution was transferred after 30 min. to a larger flask with the aid of ethanol (2 ml.). After 1 hr., excess of sodium borohydride was added to the solution to ensure the complete reduction of the keto-ester. After 2 hr., the excess of borohydride was decomposed (dil. hydrochloric acid) and the solution was treated with barium hydroxide octahydrate (30 mg.) and heated under reflux for 3 hr. It was then applied to a column of Dowex 1-X8 ion exchange resin ($OH^-$ form; 2 g.) and the column was washed thoroughly with deionised water. The dihydroxy-acid mixture was eluted with 4% sodium hydroxide (50 ml.). The eluate was acidified (conc. hydrochloric acid) and extracted continuously with ether for one day. The extract was dried ($MgSO_4$) and evaporated to give a colourless oil (935 μCi; 9·4% radiochemical yield). Paper chromatograms [systems (a) and (b)] were cut into narrow strips. The strips were moistened with liquid scintillation solution and counted in a scintillation counter. In both systems, a radiochemical purity of >99% was indicated.

*Dilution Analysis*.—Aliquot portions (7·37 μ-Ci) of the labelled dihydroxy-acid mixture were co-crystallised (ethanol) with the brucine salts[5] of (2*R*,3*S*)- and (2*S*,3*R*)-2,3-dihydroxy-2-methylbutanoic acids (2 g. each). After eight recrystallisations, the activity of the salt of the (2*S*,3*R*)-acid had fallen to 0·01% of the original. After sixteen recrystallisations, the activity of the salt of the (2*R*,3*S*)-acid reached a value corresponding to the entrainment of 32 ± 2% of the original activity; the activity remained unchanged during a further two recrystallisations.

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