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CHEMISTRY OF THE PODOCARPACEAE—V*

THE IDENTIFICATION OF MIROPINIC ACID AND ISOMIROPINIC ACID FROM *PODOCARPUS FERRUGINEUS* D. DON

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Abstract—By reinvestigation of "isomiropinic" and "miropinic" acids from the bled resin of *Podocarpus ferrugineus*, the compounds have been identified as sugiol and isopimaric acid respectively. The identity of the resin acid from *Dacrydium biforme* has been confirmed as isopimaric acid.

DURING a separation of the constituents from the bled resin of the New Zealand species *Podocarpus ferrugineus* D. Don (Maori name "Miro"), Brandt and Neubauer isolated, along with the diterpenoid resinol, ferruginol,† a resin acid fraction in smaller yield.[1] Subsequent investigation[2] showed it to be comprised of two crystalline compounds, "miropinic acid", the major constituent, and "isomiropinic acid", obtained in much lower yield. "Miropinic acid", $C_{20}H_{30}O_2$, m.p. 160°, $[\alpha]_D^{16}$ −3·6°, yielded, on catalytic hydrogenation in neutral solvent, two isomeric dihydro-acids each of which in turn gave rise to different saturated tetrahydro-acids together with a small amount of a third dihydro-isomer, on further hydrogenation in acetic acid solution. "Miropinic acid" formed a liquid methyl ester and when dehydrogenated with selenium it yielded pimanthrene. Further, the melting point of "miropinic acid" was undepressed by an unnamed acid isolated by Hosking and Brandt from the related species *Dacrydium biforme* Pilger[3] and also by Hosking from *Dacrydium kirkii* F. Muell.[4] From these results they concluded that "miropinic acid" was either identical or stereoisomeric with the acids from *D. biforme* and *D. kirkii* and might be identical with cryptopimaric acid, m.p. 159–161°, $[\alpha]_D^{16·3}$ −20·83°, $[\alpha]_D^{17·5}$ −18·99°, from *Cryptomeria japonica*.[5] Jeger and Brossi[6] subsequently reported that "miropinic acid" was identical with isopimaric (isodextropimaric) acid‡ by direct comparison of the acids and their methyl esters and by comparison of the infra-red spectra of the methyl esters.

* Part IV. L. H. Briggs, R. C. Cambie, R. N. Seelye and A. D. Warth, *Tetrahedron* **7**, 270 (1959).

† Bredenberg (ref 12) has shown that this compound contains small amounts of Δ^9-dehydroferruginol.

‡ In accord with E. Wenkert and J. W. Chamberlin, *J. Amer. Chem. Soc.* **81**, 688 (1959); Le Van Thoi, *C.R. Acad. Sci., Paris* **247**, 1343 (1958) and O. E. Edwards and R. Howe, *Chem. & Ind.* 537 (1959) the simplified name is used throughout.

[1] C. W. Brandt and L. G. Neubauer, *J. Chem. Soc.* 1031 (1939).
[2] C. W. Brandt and L. G. Neubauer, *J. Chem. Soc.* 683 (1940).
[3] J. R. Hosking and C. W. Brandt, *Chem. Ber.* **68**, 1313 (1935).
[4] J. R. Hosking, *New Z. J. Sci. and Tech.* **19**, 208 (1937).
[5] S. Keimatsu, T. Ishiguro and G. Fukuri, *J. Pharm. Soc. Japan* **57**, 69 (1937).
[6] A. Brossi and O. Jeger, *Helv. Chim. Acta* **33**, 722 (1950).

"Isomiropinic acid", $C_{20}H_{30}O_2$, m.p. 284°, $[\alpha]_D^{17}$ +21·2°, was obtained directly from the resin acid mixture and was also reported as being produced by the isomerization of "miropinic acid" with methanolic hydrogen chloride.* On catalytic reduction it gave a neutral resin, $C_{20}H_{30}O$, which was not investigated further.

In our continued investigation of the extractives of the New Zealand *Podocarpaceae* it appeared desirable to confirm Brossi and Jeger's conclusion regarding the nature of "miropinic acid". It may be pointed out that Brossi and Jeger did not in reality compare isopimaric and "miropinic" acids. What they did compare with the former acid was the unnamed resin acid from *D. biforme* which they assumed to be identical with "miropinic acid" from *D. ferrugineus*. In view of the difficulties encountered in the separation of pure resin acids this assumption is not necessarily valid. As Brandt and Neubauer realized, without a determination of the optical acitvity of the acid from *D. biforme*, "miropinic acid" could be a stereoisomer of it, despite the fact that no depression in melting point was observed on admixture. Furthermore, the infra-red spectra of the methyl esters published by Brossi and Jeger,[6] although very similar, are not coincidental at all points and could possibly be those of stereoisomers. During our investigations of the diterpene hydrocarbons[7,8] we have found that the stereoisomeric compounds phyllocladene, mirene and kaurene possess infra-red spectra which show only small differences in the "finger print" region, of order comparable to those exhibited by the above methyl esters.[6] If in fact "miropinic acid" is identical with isopimaric acid it also appeared necessary to explain if possible, the isomeric dihydro- and tetrahydro- derivatives of "miropinic acid", the isomerization of "miropinic acid" to "isomiropinic acid" and to reinvestigate the nature of "isomiropinic acid" itself, and also its hydrogenated product. For example, from the method of isolation of "isomiropinic acid" viz. by precipitation from a sodium hydroxide solution with carbon dioxide and from the insolubility of the hydrogenated product in aqueous sodium hydroxide it appeared doubtful that it was in fact a resin acid.

Fortunately, small samples of the original "miropinic acid" and "isomiropinic acid" isolated by Brandt and Neubauer from *Podocarpus ferrugineus*[2] were available for further study. Three recrystallizations of "isomiropinic" acid from glacial acetic acid raised the melting point to 292–293° and analysis favoured the formula, $C_{20}H_{28}O_2$, rather than $C_{20}H_{30}O_2$, as proposed by Brandt and Neubauer. Furthermore, it formed a crystalline acetate, m.p. 164–165°, slowly reacted with 2,4-dinitrophenylhydrazine reagent and possessed an ultra-violet spectrum (maxima at 233 mμ and 285 mμ) markedly different from that of isopimaric or pimaric acid.[9] These properties are in excellent agreement with those of the phenolic ketone, sugiol, isolated from the heartwood of *Podocarpus dacrydioides* A. Rich. (Part IV) and by Brandt and Thomas from the related species, *Dacrydium cupressinum* Soland.[10] The identification was confirmed by direct comparison and by identical infra-red spectra.

"Miropinic acid" was purified via the butanolamine salt and regeneration of the free acid by Harris and Sanderson's method.[9] It then had m.p. 162–164°, zero rotation

* It is assumed that the melting point of 184° recorded for the compound isolated in this manner is a misprint for 284°.

[7] L. H. Briggs, B. F. Cain, B. R. Davis and J. K. Wilmshurst, *Tetrahedron Letters* No. 8, 8, 13 (1959).
[8] L. H. Briggs, B. F. Cain and R. C. Cambie, *Tetrahedron Letters* No. 8, 17 (1959).
[9] G. C. Harris and T. F. Sanderson, *J. Amer. Chem. Soc.* **70**, 2079 (1948).
[10] C. W. Brandt and B. R. Thomas, *New Z. J. Sci. Tech.* **33B**, 30 (1951); *J. Chem. Soc.* 2442 (1952).

and an infra-red spectrum identical with that of isopimaric acid.* The infra-red spectra of the respective methyl esters were also identical.

Isopimaric and pimaric acid co-occur in other plants[9] but, in this case, pimaric acid could not be detected in the mother liquors from the purification of isopimaric acid.

In contrast to the two dihydro derivatives obtained by Brandt and Neubauer, partial hydrogenation of the purified acid under the conditions of Edwards and Howe[11] led to a dihydro derivative which had physical constants in excellent agreement with their dihydroisopimaric acid.

With the identification of "isomiropinic acid" and "miropinic acid" established as sugiol and isopimaric acid respectively, it now became possible to clarify some of the points raised earlier. The neutral resin, $C_{20}H_{30}O$, obtained by Brandt and Neubauer on catalytic hydrogenation of sugiol was apparently ferruginol formed by reduction of the keto-group to 9-oxyferruginol, followed by dehydration to the dehydro derivative and catalytic hydrogenation of the double bond. Bredenberg[12] has shown that reduction of sugiol with aluminium isopropoxide leads to a high yield of the dehydro derivative as a result of the ready dehydration of the intermediate 9-oxyferruginol. The identity of the neutral product was confirmed by repetition of the catalytic reduction of sugiol and isolation of ferruginol as its crystalline acetate.

Despite the fact that it has been shown that the original sample of "miropinic acid" was impure and that in our hands only a single dihydro derivative was obtained from the purified compound, the recent work of Edwards and Howe on the stereochemistry of the isomeric pimaric acids,[11,13] allows for a possible explanation of Brandt and Neubauer's three isomeric dihydro- and two isomeric tetrahydro-derivatives. These workers have shown that rigorously purified dihydroisopimaric acid has m.p. 173–175°, $[\alpha]_D$ −7°, while tetrahydroisopimaric acid has m.p. 172–173°, $[\alpha]_D$ +24°. However, it was observed that hydrogenation is accompanied by double bond migration to the Δ^{13}-dihydro compound, but that it was not possible to form the pure isomer on the catalyst surface. The physical constants of "α-dihydromiropinic acid", m.p. 176°, $[\alpha]_D$ −10·5°, and "α-tetrahydromiropinic acid", m.p. 170°, $[\alpha]_D$ +15·2°, correspond reasonably well with those of dihydro- and tetrahydroisopimaric acids, respectively, and are probably almost pure compounds. The physical constants of "β-dihydromiropinic acid", m.p. 115°, $[\alpha]_D$ +23·2°, suggest that it may be a mixture of isopimaric and Δ^{13}-isopimaric acids (m.p. 106–107°, $[\alpha]_D$ +113°), probably corresponding to the 1 : 1 synthetic mixture m.p. 118–120°, $[\alpha]_D$ +58°, of Edwards and Howe, with dihydroisopimaric as impurity. "γ-dihydromiropinic acid" could be a similar mixture or a mixture of dihydroisopimaric acid and Δ^{13}-dihydropimaric acid (m.p. 107–108°, $[\alpha]_D$ +74°[11]) while "β-tetrahydromiropinic acid", m.p. 170°, $[\alpha]_D$ +30·5°, may well be mainly tetrahydroisopimaric acid containing a trace of Δ^{13}-dihydroisopimaric acid as impurity.

Finally, purification of the resin acid from *Dacrydium biforme*, m.p. 148–150°, via the butanolamine salt, gave a pure compound, m.p. 162–164°, $[\alpha] \pm 0$, the infra-red

* See also comparisons of the spectra of the pimaric acids [H. H. Bruun, *Paper and Timber, Finland* **38**, 557 (1956); **39**, 221 (1957); H. H. Brunn, I. Fischmeister and E. Stenhagen, *Acta Chem. Scand.* **13**, 379 (1959)].

[11] O. E. Edwards and R. Howe, *Canad. J. Chem.* **37**, 760 (1959).
[12] J. B. Bredenberg, *Acta Chem. Scand.* **11**, 932 (1957).
[13] O. E. Edwards and R. Howe, *Chem. & Ind.* 629 (1958).

spectrum of which was identical with that of isopimaric acid, thus confirming Brossi and Jeger's identification of this compound.

EXPERIMENTAL

Analyses were by Dr. A. D. Campbell, University of Otago, New Zealand. Infra-red spectra were measured as KBr discs with a Beckman IR2 instrument (NaCl prism) and ultra-violet spectra for EtOH solutions with a Beckman DU instrument.

Sugiol. Three recrystallizations of "isomiropinic acid", m.p. 284°, from glacial acetic acid (charcoal) gave sugiol as long colourless needles, m.p. and mixed m.p. 292–293°, $[\alpha]_D^{20}$ +22·6° (*c* 1·2 in dioxan), $[\alpha]_D^{20}$ +34·2° (*c* 1·5 in pyridine) (Found: C, 80·0; H, 9·2; Calc. for $C_{20}H_{28}O_2$: C, 80·0; H, 9·4%). λ_{max} 233 mμ (log ε 4·21) and 285 mμ (log ε 4·14), (identical infra-red spectrum). The acetate, prepared by the use of acetic anhydride–pyridine (100°; 2 hr), formed needles, m.p. and mixed m.p. 164–165°, from aqueous methanol.

Ferruginol. Sugiol (500 mg) was catalytically hydrogenated by the method of Brandt and Neubauer for "isomiropinic acid"[2] for 2 hr at room temp. Filtration and removal of solvent from the filtrate, followed by distillation of the oil obtained, gave ferruginol (210 mg), b.p. 180–200°/0·5 mm as a pale yellow resin. Acetylation by the method of Brandt and Neubauer[1] gave ferruginyl acetate (200 mg) which crystallized from ethanol (charcoal) as colourless rods, m.p. and mixed m.p. 80–81°.

Isopimaric acid. Combined crude samples of "miropinic acid" (600 mg) in methyl acetate (20 cc) were treated with 2-amino-2-methylpropan-1-ol (250 mg) in the same solvent (5 cc) according to the method of Harris and Sanderson.[9] After three recrystallizations as flat rods from a large volume of methyl acetate, the salt had m.p. 194–196°, with preliminary sintering, $[\alpha]_D^{20}$ ±0° (*c* 2·5 in EtOH). The isopimaric acid liberated from the salt, after four recrystallizations from 50% aqueous ethanol had m.p. and mixed m.p. 162–164°, $[\alpha]_D^{20}$ ±0° (*c* 1·31 in EtOH) (Found: C, 79·7; H, 9·6. Calc. for $C_{20}H_{30}O_2$: C, 79·4; H, 10·0%). The infra-red and ultra-violet spectra were each identical with the respective spectra of an authentic sample of isopimaric acid with the same physical constants.* The methyl ester prepared by the use of diazomethane had m.p. and mixed m.p. 60–61° (identical infra-red spectrum).

Isopimaric acid from Dacrydium biforme. A sample of resin acid, m.p. 148–153°, from *D. biforme.* was purified by the above method. After two recrystallizations from 50% aqueous ethanol it had m.p, and mixed m.p. 162–164°, $[\alpha]_D^{20}$ ±0° (identical infra-red spectrum).

Dihydroisopimaric acid. A solution of isopimaric acid (300 mg) in ethanol (20 cc) was hydrogenated in the presence of pre-reduced palladium charcoal catalyst (10%; 250 mg) at room temp for 10 min. Purification by chromatography on silica gel by Edward and Howe's method gave dihydroisopimaric acid (230 mg) as rods, m.p. 172–174°, $[\alpha]_D^{20}$ −7·6° (*c* 1·23 in EtOH) (Found: C, 79·1; H, 10·6. Calc. for $C_{20}H_{32}O_2$: C, 78·9; H, 10·5%).

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