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Two-dimensional INADEQUATE ^{13}C NMR Studies of Maleopimaric Acid, the Diels–Alder Adduct of Levopimaric Acid and Maleic Anhydride, and of Abietic Acid

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Two-dimensional INADEQUATE ^{13}C NMR was used to establish firmly the carbon–carbon connectivities present in abietic acid and maleopimaric acid, i.e. the adduct of levopimaric acid and maleic anhydride. This information leads to unambiguous assignments for the 20 and 24 carbon resonances of abietic acid and maleopimaric acid, respectively. Proton–carbon chemical shift correlation and NOE experiments further revealed the three-dimensional structure of maleopimaric acid.

KEY WORDS Rosin Maleopimaric acid INADEQUATE

INTRODUCTION

Rosin or colophonium is a solid resinous material that occurs in the oleoresin from pine trees. The resin acid composition of rosin has been extensively investigated,[1,2] particularly since the introduction of chromatographic methods. The nine common resin acids have a similar structure,[1,2] the molecular formula $C_{20}H_{30}O_2$ and belong to the class of tricyclic diterpenes. A subdivision can be made into those resin acids where two or three double bonds are present in conjugated positions (abietadienoic acids), i.e. abietic, levopimaric, palustric, neoabietic and dehydroabietic acid (aromatic), and those resin acids where the two double bonds are in non-conjugated positions with respect to each other, i.e. the pimaradienoic and isopimaradienoic acids (pimaric, sandaropimaric, isopimaric and $\Delta^{8,9}$-isopimaric). Abietic acid (**1**) (Fig. 1) is the most common of these resin acids by virtue of its ready formation by acid-catalysed isomerization of rosin. Levopimaric acid (**2**), on the other hand, is the key compound in modified rosins because of its ability to form a Diels–Alder adduct with maleic anhydride[2,3b,4] called maleopimaric acid (**3**).

Structural studies of these resin acids using NMR are scarce; however, partial ^1H NMR assignments have been reported for all acids[5] or their methyl esters.[6] Moreover, ^{13}C NMR assignments have recently been reported for some abietadienoic acids, i.e. abietic[7] and dehydroabietic acid[8] and for all four pimaradienoic and isopimaradienoic acids or their methyl esters.[9–11] For levopimaric acid and its Diels–Alder adduct (**3**), only partial and tentative ^1H NMR assignments exist.[3a,4,6]

The chemistry of rosin based resins has been discussed[12] in an extensive study using ^{13}C NMR as a tool for molecular structure elucidation. Assignments of all olefinic and aliphatic methine and methyl resonances were established for all nine resin acids present in rosin, mainly based on the results obtained via DEPT experiments[13] on rosin samples from different pine trees.

Because of the commercial importance of (modified) rosins (manufactured by, among others, DSM Resins, Zwolle, The Netherlands) and the structural relationship to other resin acids and terpenes, it is worthwhile to assign unambiguously the ^{13}C NMR resonances of the two important compounds, i.e. abietic acid (**1**) and maleopimaric acid (**3**). In this work, several 1D and 2D NMR techniques were used to assign the various resonances. The 2D INADEQUATE technique was used to determine the carbon–carbon connectivities. Consequently, the proton assignment was established via ^1H–^{13}C heteronuclear chemical shift correlation and NOE-difference experiments.

EXPERIMENTAL

Abietic acid was obtained from Fluka and was purified in the usual manner.[14] An independent batch of ultrapure abietic acid was kindly given by Abieta Chemie (F.R.G.). Maleinized rosin was obtained via the reaction of a mixture of Portuguesian gum rosin (1 mol) and maleic anhydride (1.08 mol) for 1 h at 200 °C.

The purified levopimaric acid–maleic anhydride adduct was obtained from the crude reaction mixture

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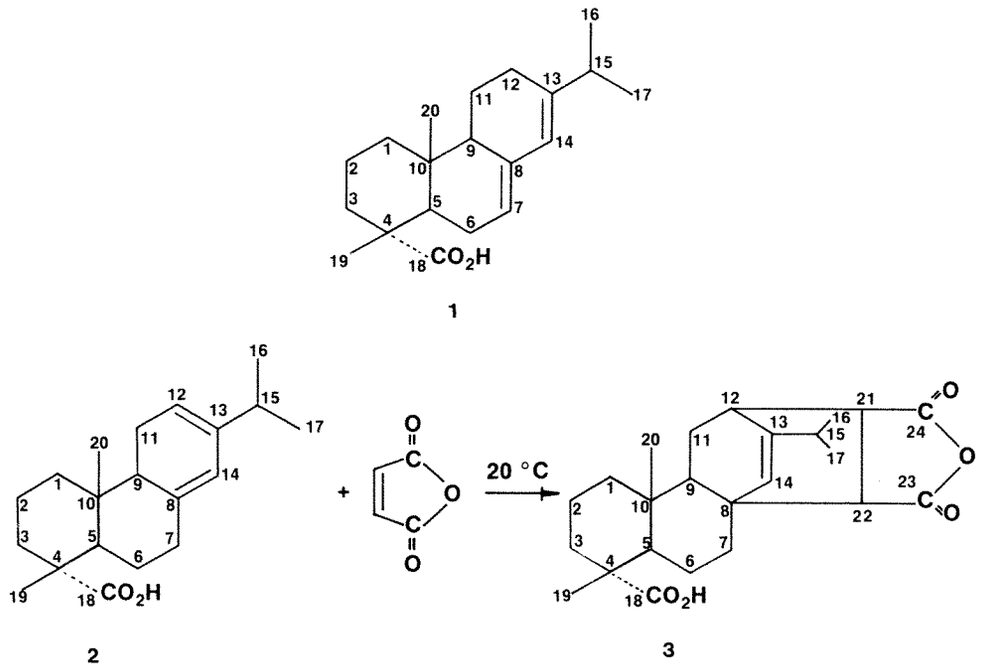

1 2 3

Figure 1. Structures and numbering scheme for abietic (**1**), levopimaric (**2**) and maleopimaric acid (**3**).

by dissolving the latter in toluene and precipitating the adduct by the addition of *n*-heptane.[3b]

The ^{13}C 2D INADEQUATE spectra of abietic acid and maleopimaric acid were recorded on a Bruker WM 250 spectrometer, operating at 62.89 MHz (1.0 M in $CDCl_3$, 10 mm tube, 313 K). The pulse sequence of Bax and co-workers[15,16] was used with a 32 + 32 transient phase cycle for *N*-type selection in F_1. A τ value of 7 ms was taken for this sequence [$\tau = (2n + 1)/4J(cc)$, $n = 0$], $F_1 = \pm 3145$ Hz, $F_2 = 3145$ Hz, recycle delay 1.0 s; 1356 scans were accumulated for each of the 96 FIDs with 2 K data points. Gaussian multiplication was applied in both dimensions. To reduce the T_1 relaxation time, 0.03 M $Cr(acac)_3$ was added to the solution. For the ^1H–^{13}C heteronuclear chemical shift correlation[17] spectrum of maleopimaric acid, 128 experiments were performed with 32 scans each. Delays were 3.85 and 1.92 ms, with a relaxation delay of 1 s; $F_1 = 2000$ Hz, $F_2 = 13\,888.9$ Hz. The final data matrix was 256 × 4 K, and no window functions were applied. For the long-range ^1H–^{13}C correlation experiment, delays of 41.8 and 20.8 ms were used.

RESULTS AND DISCUSSION

The contour plot of the 2D INADEQUATE experiment for maleopimaric acid (**3**) is given in Fig. 2; ^{13}C pairs are circled and numbered according to their connectivities. Only the aliphatic region was observed; the assignments for the olefinic and carbonyl carbon atoms were easily obtained either from their chemical shift values or from proton–carbon shift correlation spectra[17] optimized for both $^1J(CH)$ and long-range $J(CH)$.[18] Thus, C-23 and C-24 could be differentiated because C-24 had a clear correlation with H-12 in a long-range ^1H–^{13}C experiment optimized for $^3J(CH)$.

The INADEQUATE experiment was optimized for sp^3–sp^3 connectivity ($\tau = 7$ ms). Adding 0.03 M $Cr(acac)_3$ to the $CDCl_3$ solution of the adduct **3** reduced the T_1 relaxation times. This allowed us to use a short recycle delay. Nineteen of the total of 20 connectivities in the aliphatic region were found from the INADEQUATE spectrum of **3** shown in Fig. 2. Only connectivity 1–10 is missing. Apparently these carbon atoms have a very strong AB coupling pattern.[19] All assignments and chemical shift values are given in Table 1, and comparison with our earlier data shows that several assignments must be changed.[12]

The 2D INADEQUATE spectrum of abietic acid (**1**) was also recorded for comparison purposes (Fig. 3). This allowed the unambiguous assignment of all 20 signals, which proved to be in full agreement with the data of Smith.[20]

Table 1. ^{13}C NMR Chemical shift data[a] for abietic acid (1) and maleopimaric acid (3)

Carbon	$\delta^{13}C$ (1)	$\delta^{13}C$ (3)	Carbon	$\delta^{13}C$ (1)	$\delta^{13}C$ (3)
1	38.37	37.97	13	145.12	148.07
2	18.11	16.89	14	122.54	125.12
3	37.27	36.74	15	34.90	32.69
4	46.40	46.77	16	20.89	19.91[b]
5	45.01	49.05	17	21.42	20.50[b]
6	25.66	21.62	18	185.20	184.86
7	120.48	34.76	19	16.71	16.40
8	135.56	40.37	20	14.05	15.42
9	51.05	53.25	21		45.58
10	34.54	37.51	22		53.03
11	22.55	27.18	23		172.64
12	27.49	35.64	24		170.87

[a] 0.3 M in $CDCl_3$ at 303 K.
[b] May be interchanged.

The ^1H NMR assignments and chemical shifts for **3** are given in Table 2. These assignments were made via a 250-MHz ^1H spectrum and a ^1H–^{13}C heteronuclear chemical shift correlation experiment.[17] Although the reaction between levopimaric acid (**2**) and maleic anhydride can theoretically lead to four isomers, only one

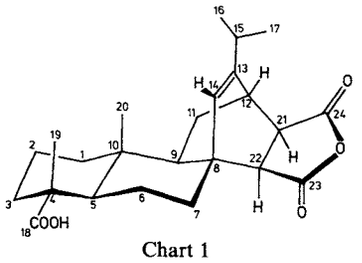

Chart 1

Table 2. ^1H NMR Chemical shift data[a] for maleopimaric acid (3)

Protons	δ^1H	Protons	δ^1H	Protons	δ^1H
H-1eq	1.41	H-7ax	1.70	16-CH_3	0.97[b]
H-1ax	0.95	H-9	1.40	17-CH_3	1.00[b]
2-CH_2	1.47	H-11eq	1.66	19-CH_3	1.16
3-CH_2	1.62	H-11ax	1.23	20-CH_3	0.59
H-5	1.76	H-12	3.11	H-21	3.08
6-CH_2	1.38	H-14	5.53	H-22	2.72
H-7eq	2.52	H-15	2.25		

[a] Obtained from the 250-MHz ^1H NMR spectrum of **3** in 0.3 M $CDCl_3$ or from the 62.89-MHz 2D ^{13}C–^1H correlation spectrum of the same solution.
[b] May be interchanged.

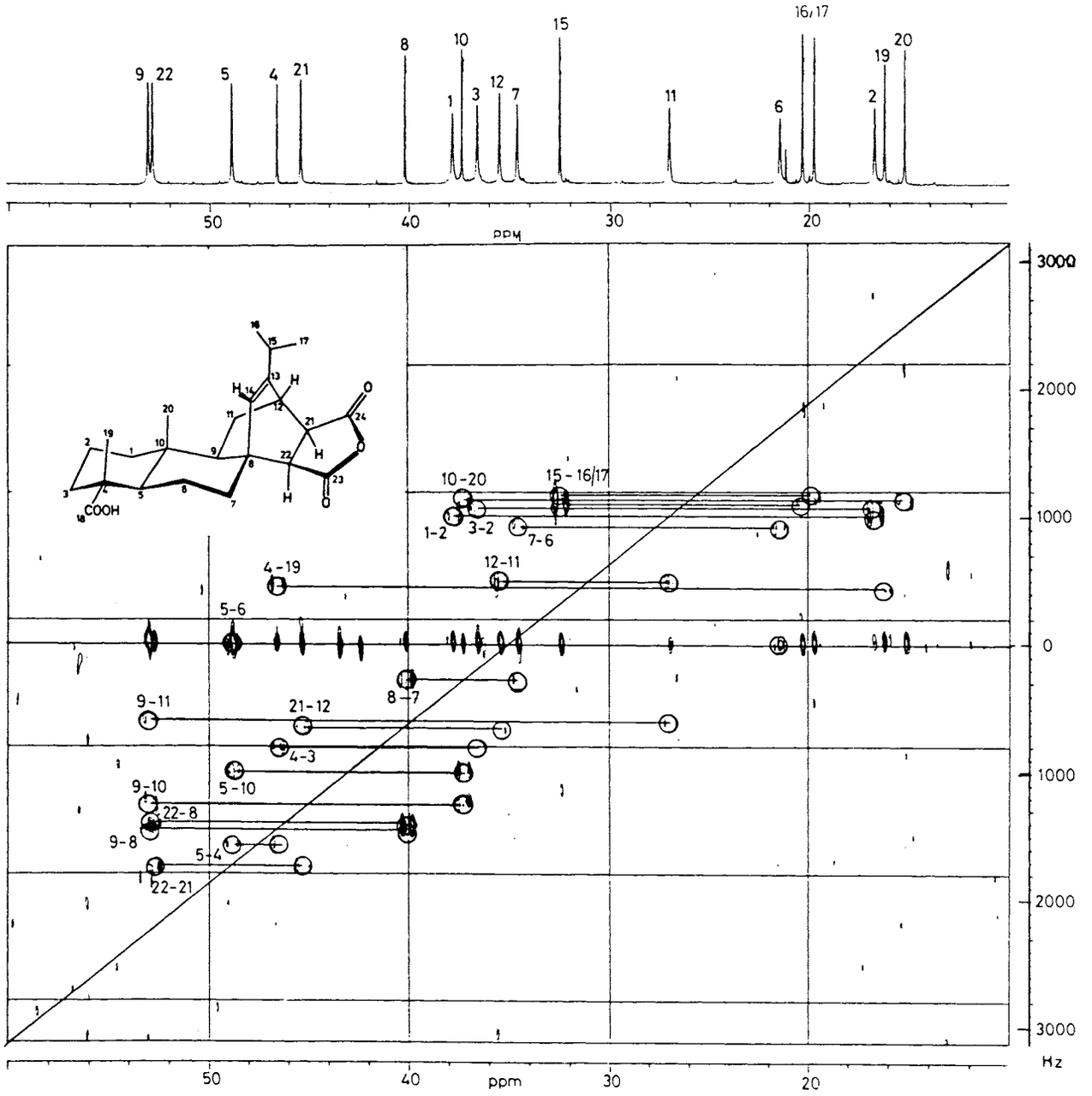

Figure 2. 62.89-MHz ^{13}C NMR 2D INADEQUATE contour plot of **3** (1.0 M in $CDCl_3$, 313 K). 0.03 M $Cr(acac)_3$ was added. The 1D ^{13}C NMR spectrum (partial) is shown above.

isomer is formed in high yield. Therefore, we decided to perform NOE-difference experiments using the multiple irradiation technique[21,22] in order to establish which isomer is actually formed. Meyer and Hoffman[4] stated that the structure can be described as depicted in Chart 1, because of the shielding effects observed for the protons of the 20-Me group.

The results of the NOE-difference measurements are given in Table 3. NOE effects are observed, for example, between H-20 and H-14 and between H-22 and H-9ax. These observations are particularly important since they confirm the presence of H-14 above and of H-22 below the plane formed by the four rings. This excludes the two isomers which are formed by dienophile attack of maleic anhydride from above on the diene **2** because this results in the positioning of H-14 below and H-22 above the rings. The fourth possible isomer, which in the same way as **3** would result from dienophilic attack from below, but with the anhydride ring pointing down-

Table 3. NOE effects observed for 3[a]

Proton irradiated	Proton observed
H-7eq	H-14, H-7ax, H-6eq
H-20	H-14, H-19, H-6ax, H-2ax
H-14	H-7eq, H-15, H-16/17, H-20
H-12	H-15, H-11eq, H-11ax, H-21
H-22	H-7ax, H-9ax, H-21
H-21	H-12, H-22

[a] All these experiments were obtained after adding a few drops of C_6D_6 to the $CDCl_3$ solution, which then gave a clear separation of the absorption signals of H-12 and H-21.

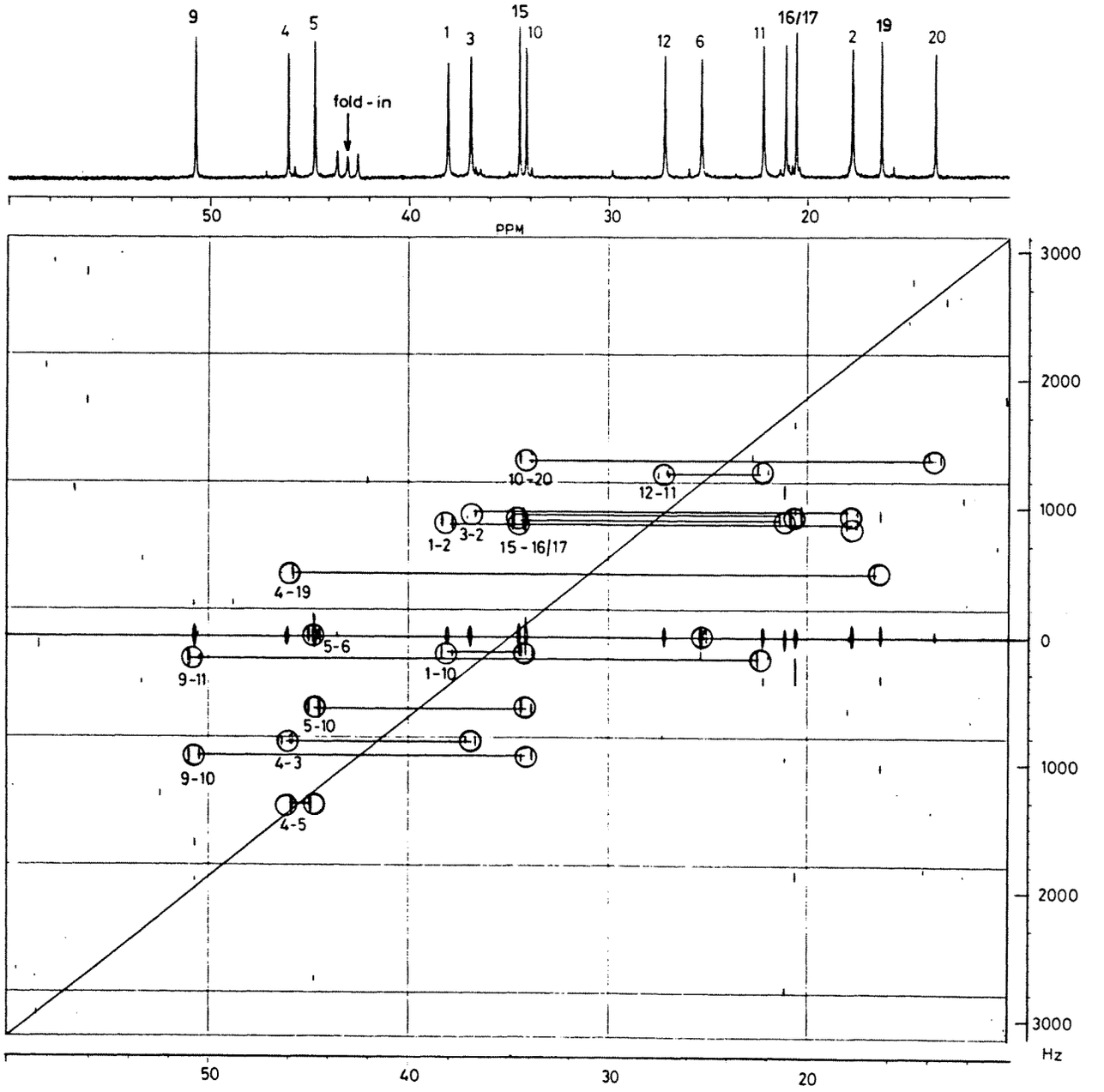

Figure 3. 62.89-MHz ^{13}C NMR 2D INADEQUATE contour plot of **1** (1.0 M in $CDCl_3$, 313 K). 0.03 M $Cr(acac)_3$ was added. The 1D ^{13}C NMR spectrum (partial) is shown above.

wards and H-21/H-22 pointing sideways, can also be excluded on the basis of the observed NOEs.

In conclusion, a combination of 1D and 2D NMR techniques allows the unambiguous assignment of all proton and carbon resonances (except for 16-Me and 17-Me which may be interchanged), as well as the elucidation of the three-dimensional structure of maleopimaric acid (**3**). Some previously published[12] carbon chemical shifts have now been corrected on the basis of a 2D INADEQUATE experiment.

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