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^{13}C NUCLEAR MAGNETIC RESONANCE STUDIES OF SESQUITERPENES, ANISATIN AND NEOANISATIN

THE STRUCTURE OF ANISATINIC ACID, A NOVEL ISOMERIZATION PRODUCT OF ANISATIN

SHUNICHI MANABE, KAZUMASA WAKAMATSU, YOSHIMASA HIRATA and KIYOYUKI YAMADA*
Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya, Japan

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Abstract—^{13}C NMR spectra are reported for derivatives of toxic sesquiterpenes, anisatin (I) and neoanisatin (II). Chemical shifts for each compound have been assigned on the basis of off-resonance decoupling experiments, known chemical shift rules, and comparison of the spectra among the compounds examined. Toxic anisatin (I) is known to isomerize under mild conditions to a non-toxic compound, anisatinic acid. The structure of anisatinic acid has been determined unambiguously to be IIIa by the ^{13}C NMR spectral analysis of a derivative 8 of anisatinic acid. Some aspects of the substituent effects on the ^{13}C chemical shifts obtained in the present investigation are described.

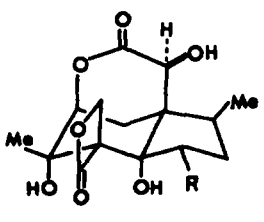
Previously two sesquiterpenes, anisatin and neoanisatin were isolated as toxic components of *Illicum Anisatum* L. from the seeds of the plant, and their structures were elucidated as I and II, respectively.[1] Anisatin (I) was found to exhibit unusual reactivities because of the presence of many functional groups in the close proximity within the molecule.[1-3] As one of the unique reactions of anisatin (I), the base-catalyzed isomerization of toxic anisatin to a non-toxic compound, anisatinic acid can be cited.[2,4] From the viewpoint of the structure-toxicity relationship concerning anisatin, it is important to establish the structure of anisatinic acid unambiguously.

It is known that anisatin (I) is very stable under strongly acidic conditions, whereas it undergoes facile isomerization simply by heating in the MeOH solution or by the action of alkali such as $NaHCO_3$ or ammonia at room temperature, resulting in the fomation of anisatinic acid.[2,4] Of the two possible structures (IIIa and IIIb) presented for anisatinic acid, the former structure (IIIa) was deduced to be the actual one on the basis of the findings that no bands appeared in the CO region of the IR spectrum of the Na salt of anisatinic acid and that only a negative plain curve was observed in the ORD measurement of anisatinic acid.[2]

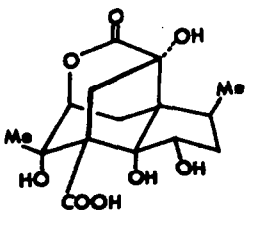
Recent advances in the ^{13}C NMR spectroscopy have made it possible to apply successfully this valuable tool to the structural studies of complex natural products.[5] We have examined the ^{13}C NMR spectra of derivatives of anisatin (I), neoanisatin (II) and anisatinic acid in order to establish the structure of anisatinic acid unambiguously, and the results are described in the present paper.

^{13}C NMR *chemical shift assignments for derivatives of anisatin* (I) *and neoanisatin* (II)

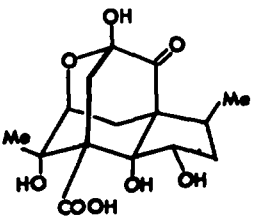
Before discussing the structural problem of anisatinic acid based on the ^{13}C NMR spectroscopy, it is a prerequisite to assign the ^{13}C resonances to individual carbons for derivatives of anisatin (I) and neoanisatin (II). The ^{13}C NMR spectra of the seven derivatives (1–7)[1,3] of anisatin (I) and neoanisatin (II), among which 2 was newly prepared in the present study were obtained, and the assignments of the resonances to individual carbons of each compound were made, the results being summarized in Table 1. The ^{13}C NMR chemical shift assignments shown in Table 1 were based on the single frequency off-resonance decoupling techniques, application of known chemical shift rules[6-8] including acetylation shifts,[7,9-11] and comparisons of the spectra among the compounds examined. In connection with the ^{13}C NMR spectrum of a derivative 8 of anisatinic acid mentioned later, special attention was given to the ^{13}C chemical shift assignments for 2. The discussion, therefore is made mainly on 2.



I: R = OH (anisatin)
II: R = H (neoanisatin)



IIIa



IIIb

Scheme 1.

Table 1. ^{13}C Chemical shifts for the derivatives of anisatin and neoanisatin[a,b,c]

carbon	1	2	3	4	5	6	7	8
1	36.7(d)	36.6(d)	36.2(d)	37.2(d)	31.2(d)	30.2(d)	33.3(d)	36.0(d)
2	37.3(t)	37.4(t)	37.3(t)	30.1(t)	34.8(t)	43.9(t)	28.7(t)	37.3(t)
3	73.4(d)	74.4(d)	73.7(d)	33.3(t)	73.2(d)	204.6(s)	32.5(t)	74.5(d)
3a	84.6(s)	82.4(s)[d]	82.3(s)[d]	83.3(s)[e]	79.4(s)[e]	72.5(s)	80.4(s)[e]	86.7(s)
4	64.0(s)	65.0(s)	64.8(s)	66.0(s)	66.6(s)	64.2(s)	67.9(s)	57.6(s)
5	74.3(s)	82.4(s)[d]	82.3(s)[d]	83.1(s)[e]	79.7(s)[e]	83.2(s)	81.4(s)[e]	83.4(s)
6	81.5(d)	76.3(d)	75.5(d)	75.4(d)	79.0(d)	81.5(d)	78.6(d)	77.4(d)
7	26.3(t)	26.5(t)	26.7(t)	27.3(t)	29.4(t)	29.5(t)	29.7(t)	28.9(t)
7a	49.4(s)	49.2(s)	49.7(s)	50.2(s)	56.9(s)	57.8(s)	59.2(s)	55.2(s)
8	13.3(q)	13.4(q)	13.2(q)	13.2(q)	13.4(q)	12.0(q)	12.8(q)	12.4(q)
9	167.8(s)	166.8(s)	166.3(s)[f]	165.9(s)[f]	166.4(s)	166.1(s)	166.5(s)	170.7(s)
10	64.7(t)	64.2(t)	64.3(t)	64.8(t)	65.9(t)	66.6(t)	66.5(t)	41.5(t)
11	20.7(q)	17.7(q)	17.6(q)	17.8(q)	19.2(q)	20.6(q)	19.3(q)	19.0(q)
12	69.7(d)	69.6(d)	68.4(d)	68.8(d)				76.7(s)
13	174.0(s)	174.2(s)	167.4(s)[f]	166.6(s)[f]	174.1(s)	172.2(s)	174.7(s)	176.8(s)
$COO\underline{C}H_3$								52.4(s)

a. Spectra taken in $CDCl_3$ at 20.00 MHz on a Varian CFT-20 spectrometer; chemical shifts are in parts per million relative to tetramethylsilane.

b. The letter in parenthesis refers to the signal multiplicity obtained from single frequency off-resonance decoupling experiments: s=singlet, d=doublet, t=triplet, q=quartet.

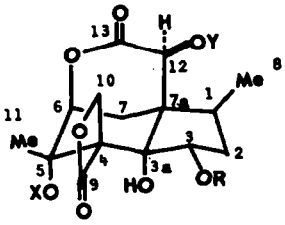
c. Data on OAc signals, δ_C 20.6-23.5 and 167.9-170.5 ppm, are not described.

d. These signals were twice as intense as those of other similar carbons.

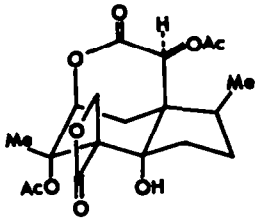
e,f. Values bearing the same superscript may be interchanged.

The ^{13}C NMR spectrum of 2 revealed, in addition to four resonances due to two acetate groups, fifteen carbon resonances, which were shown to consist of two quartets (Me), three triplets (CH_2), four doublets (methine), four singlets due to quaternary carbons, and two singlets arising from lactone CO carbons by off-resonance decoupling experiments. Two quartets at 13.4 and 17.7 ppm in 2 are assigned to C-8 and C-11, respectively, because the quartet at 20.7 ppm in 1 undergoes the upfield β shift (3.0 ppm) due to acetylation on going from 1 to 2, whereas the other quartet at 13.3 ppm in 1 was observed at 13.4 ppm in 2 without substantial change.

The three triplet resonances (26.5, 37.4 and 64.2 ppm) in 2 are for C-2, C-7 and C-10. Since C-10 is an ethereal O-substituted carbon, the C-10 resonance is expected to be shifted downfield to a large extent relative to those of C-2 and C-7, and the lowest field resonance at 64.2 ppm among the three relevant triplets is therefore assigned to C-10. The remaining two triplet resonances at 37.4 and 26.5 ppm in 2 are assigned to C-2 and C-7, respectively, on the basis of the following findings. The two triplet resonances appeared at 37.4 and 26.5 ppm in 2 are observed at 37.3 and 26.7 ppm in 3, and at 30.1 and 27.3 ppm in 4, respectively. These observations indicate that the resonance in the 26.5–27.3 ppm region remains unchanged among these compounds (2, 3, 4), while on replacing the acetoxyl group at C-3 by a hydrogen the resonance at 37.3 ppm in 3 goes upfield, being observed at 30.1 ppm in 4. This chemical shift change of the C-2 signal between 3 and 4 is in agreement with that of the C-16 signal (downfield shift by 12.4 ppm)[11] on going from cholestane to 15α-cholestanol and with the β shift value (downfield shift by *ca.* 7 ppm)[10] of acetoxyl substitution on cyclopentane derivatives, supporting the above assignment of C-2 in 2. Among the four methines (C-1, C-3, C-6 and C-12) in 2 only C-1 has no oxygen function and therefore can be assigned to the farthest upfield signal at 36.6 ppm by consideration of the chemical shift value. Comparison of other three resonances due to the O-substituted methines (C-3, C-6, C-12) in 2 with those in 1 indicates that only the signal at 81.5 ppm in 1 goes upfield (5.2 ppm) appearing at 76.3 ppm in 2, while the chemical shifts of the remaining two signals remain unchanged: the upfield shift of the signal at 81.5 ppm in 1 is clearly ascribed to the acetylation β shift and the corresponding signal at 76.3 ppm in 2 is thus assigned to C-6. The following arguments are advanced for the differentiation of the remaining methine resonances for C-3 and C-12. The only structural difference among noranisatin derivatives, 5, 6 and 7 is the functionality at C-3 and therefore the assignment of the C-3 signal among these compounds is readily made (see table): the signal at 73.2 ppm in 5 is assigned to C-3. Comparison of the spectra between 2 and 5 made it possible to assign the signal at 74.4 ppm in 2 to C-3 and, consequently the remaining signal at 69.6 ppm to C-12. The assignment of the signal at 74.4 ppm in 2 to C-3 is confirmed by comparison of the spectra of 3 and 4, since the both compounds differ solely in the C-3 functionality. The fact that the signal in the 68.4–69.7 ppm region in each spectrum of compounds, 1, 2, 3 and 4 is absent in the spectra of the compounds, 5, 6 and 7 is also a supporting evidence for the above assignment. Of the four quaternary carbons, the signals for two quaternary carbons, C-4 and C-7 should appear in the higher field region than those for other O-substituted quaternary carbons (C-3a, C-5). Since C-4 is alpha to the β-lactone CO, the low field resonance at 65.0 ppm in 2 is assigned to C-4, and hence the resonance at 49.2 ppm to C-7a. The above assignments are further confirmed by comparison of the spectra between 2 and 5: conversion of the secondary OH group at C-12 to the lactone CO results in a large



1: R = Ac, X = Y = H
2: R = X = Ac, Y = H
3: R = X = Y = Ac



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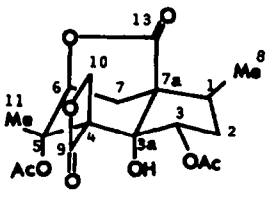

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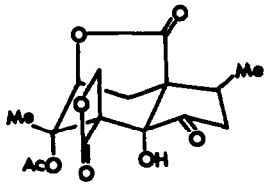

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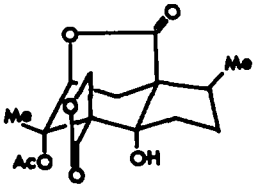

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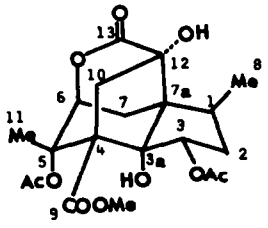

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Scheme 2.

downfield shift (7.7 ppm) of the signal at 49.2 ppm in 2, but has a small effect on the chemical shift of the signal at 65.0 ppm. The singlets for the O-substituted quaternary carbons (C-3a, C-5) show the identical chemical shifts in 2. In a series of compounds studied the chemical shifts of C-3a and C-5 are very close to each other, producing some uncertainty in their assignment: they are identical for the compound 3 and nearly identical for compounds, 4, 5 and 7. In the spectrum of 1 the signals at 74.3 and 84.6 ppm are assigned to C-5 and C-3a, respectively, since the former signal undergoes a large downfield α shift[10] (8.1 ppm) due to acetylation of the C-5 OH group on going from 1 to 2.

The two CO singlets observed at *ca.* 174 ppm and in the 166–168 ppm region of the spectra of 1 and 2 are assigned to the δ-lactone CO (C-13) and to the β-lactone CO (C-9), respectively. The assignments are based on the fact that acetylation of the C-12 OH group moves the chemical shift of the signal at *ca.* 174 ppm upfield (β shift effect) and has no significant effect on the other signal. We have observed that on acetylation of various α-hydroxy lactones the signal of the lactone CO carbon undergoes an upfield β shift.[12]

Structure of anisatinic acid

The structure of anisatinic acid, a novel isomerization product of anisatin (I) has been investigated based on the ^{13}C NMR spectral analysis of a derivative 8 of anisatinic acid.

Comparison of the spectra of 2 and 8 indicates that the remarkable structural changes occur at C-10 and C-12 by isomerization of anisatin (I) to anisatinic acid. The C-10 signal in 8 is upfield by 22.7 ppm as compared with 2 owing to the substitution of the β-lactone ethereal oxygen with a C atom, while the C-12 doublet (methine) in 2 is replaced by a downfield singlet (shift value, 7.1 ppm) in 8, revealing the increase of an alkyl substituent at C-12 of 8 as compared with 2. That the β-lactone moiety in 2 is involved in the isomerization is also indicated by the downfield shift of the resonance for the β-lactone CO carbon (166.8 ppm in 2 → 170.7 ppm in 8). Considerable changes in the chemical shifts for neighboring carbons (C-4, C-7a, C-13) of C-10 and C-12 are observed by comparison of the spectra between 2 and 8. The downfield shifts of the signals for C-7a and C-13, on going from 2 to 8 are due to the β shift effect resulting from the increase of alkyl substitution at C-12. The C-4 signal in 2 undergoes the upfield shift owing to the β shift effect caused by substitution of the acloxy group with an alkyl group at the neighboring C-10. As expected, the structural differences between 2 and 8 have almost no effects on the chemical shifts of the signals for C-1, C-2 and C-3. Definitive evidence in favor of the structure IIIa and against the structure IIIb regarding anisatinic acid is provided by the findings that the signal due to a δ-lactone CO carbon (C-13) is observed at 176.8 ppm in 8 as in 2 (174.2 ppm) and that signals corresponding to a hemiacetal carbon and a ketonic carbon are absent in 8. Thus the structure of anisatinic acid is firmly established to be IIIa.

Some aspects of substituent effect on the ^{13}C chemical shifts

Several interesting observations were made concerning the substituent effects on the chemical shifts during the course of the ^{13}C NMR spectral analysis of derivatives of anisatin (I) and neoanisatin (II).

(i) It is well known that on acetylation of a secondary or a tertiary OH group the signal of the α carbon moves downfield (1–4 ppm for secondary α carbons and *ca.* 11 ppm for tertiary α carbons) and that of the β carbon upfield (2–5 ppm).[9,10] This acetylation shifts rule is not necessarily valid for the chemical shifts changes of certain quaternary β carbons contained in compounds in the present study: comparison of the spectra of 1 and 2 indicates that on acetylation of the C-5 tertiary OH group

the C-6 and C-11 (β carbons) signals are shifted upfield in the predictable manner by 5.2 ppm and 3.0 ppm, respectively, whereas the C-4 (β carbon) signal goes downfield by 1.0 ppm. Similarly, on going from 2 to 3 the signal of C-13, one of the β carbons undergoes the upfield shift (6.8 ppm) as expected, but that of C-7a, another β carbon experiences the downfield shift by 0.5 ppm.

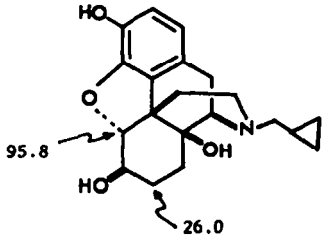
(ii) Replacement of a hydrogen or a functional group on a particular carbon by a more electronegative group is known to cause the downfield shifts for the signal of the carbon bearing the substituent (α carbon) to a large extent and for that of the neighboring carbon (β carbon) to a varying but significant extent.[7,10,11] Behaviour different from the above rule is observed as to the signals for certain β carbons of compounds in the present study. The first case is the effect on the chemical shift of the β carbon produced by substitution of a hydrogen with an acetoxyl group. On going from 4 to 3 the C-2 signal, one of the β carbons moves downfield by 7.2 ppm as expected, whereas that of C-3a, another β carbon undergoes the upfield shift (1.0 or 0.8 ppm). The similar behaviour of C-3a (β carbon) is observed (upfield shift by 1.0 or 2.0 ppm) by comparison of the spectra of 7 and 5. The substituent effect on the chemical shift of the β carbon caused by changing a methylene group to a CO group is the second case. Comparison of the spectra of 6 and 7 shows that the C-2 (β carbon) signal goes downfield to a large extent (15.2 ppm) as expected, but the C-3a (β carbon) signal contrary to the expectation moves upfield significantly (*ca.* 8 ppm). Third case is the effect on the chemical shift of the β carbon produced by changing a secondary acetoxyl group to a CO group, which is illustrated by comparison of the spectra of 5 and 6: the C-2 (β carbon) signal is markedly shifted downfield by 9.1 ppm, while the C-3a (β carbon) signal goes upfield by *ca.* 7 ppm.

In all the cases described in (ii), on replacing a hydrogen or a functional group at C-3 by a more electronegative group, the chemical shift of the C-3a (β carbon) signal moves in the reverse direction expected from the general rule mentioned above. Though the reason(s) for this apparent anomaly is not clear, one possible explanation is as follows. Replacement of a certain group at C-3 by a more electronegative one must make the β carbons (C-2 and C-3a) more deshielded, but the presence of the OH group at C-3a would decrease or in some cases compensate in excess the deshielding effect produced by the C-3a substituent. It should be noted that the similar trend concerning the signal for the O-substituted β carbon is observed in the spectra of morphine derivatives (9, 10),[13] and of neurolenins (11, 12).[14]

EXPERIMENTAL

The m.ps are uncorrected. The IR spectrum was taken with a JASCO Model IRS spectrometer. The ^{1}H NMR spectrum was obtained on a NEVA NV-21 (90 MHz) instrument. The low resolution mass spectrum was determined on a Hitachi RMU-6C mass spectrometer equipped with a direct inlet system. The ^{13}C NMR spectra were recorded on a Varian CFT-20 spectrometer operating at 20.00 MHz in the Fourier transform mode. FT measurement conditions were as follows: spectral width, 4505 Hz; pulse flipping angle, *ca.* 25°; acquisition time, 0.909 sec; number of data points, 8192. The chemical shifts are reported in ppm downfield from internal TMS and are estimated to be accurate ±0.1 ppm. The spectra were determined as 0.18–0.24 M solns in $CDCl_3$ in 8-mm tubes at 27°. Silica gel 60 F_{254} (No. 5715) and 60 PF_{254} (No. 7747) (E. Merck, A.G., Germany) were used for tlc: thickness employed was 1.50 mm for preparative tlc.

Anisatin diacetate 2. A mixture of 103 mg of 1[1,3] in Ac_2O (2.1 ml)-pyridine (0.7 ml) was stirred at room temp for 10 hr and evaporated *in vacuo*. The oily residue was separated by preparative tlc [EtOAc–$CHCl_3$ (3:1)] to give 1 (16 mg), 2 (98 mg), and 3[1] (25 mg, 23%). Recrystallization of crude 2 from benzene-hexane afforded plates of 2 (78 mg, 80%) containing 1/3 mole



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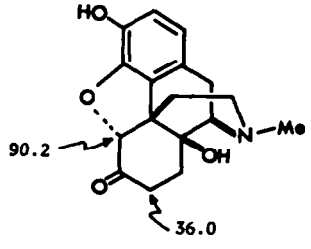

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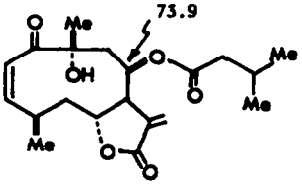

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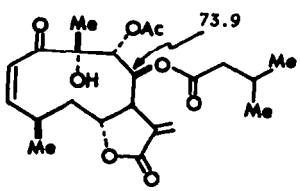

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Scheme 3.

C_6H_6 as solvent of crystallization (^{1}H NMR analysis), m.p. 58–60°; IR ($CHCl_3$) 3520, 1832, 1739 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.05 (3H, d, J = 7.0 Hz, H-8), 1.87 (3H, s, H-11), 2.04 (3H, s, AcO), 2.09 (3H, s, AcO), 4.13 (1H, s, H-12), 4.21 (2H, ABq, J = 6.5 Hz, $\Delta\nu_{AB}$ = 16 Hz, H-10), 5.69 (1H, dd, J = 2.0, 4.5 Hz, H-6), 5.93 (1H, dd, J = 6.0, 9.0 Hz, H-3); Mass 412 (M^+), 394, 352. (Found: C, 57.55; H, 6.43. $C_{19}H_{24}O_{10} \cdot 1/3C_6H_6$ requires: C, 57.53; H, 5.98%).

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