Carbon-13 Nuclear Magnetic Resonance Spectra of Anti-inflammatory Drugs: Phenylbutazone, Oxyphenbutazone and Indomethacin

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The ^{13}C nmr chemical shifts of phenylbutazone, oxyphenbutazone, indomethacin and indole-3-acetic acid are reported. The assignments of various carbon resonances are made on the basis of the substitution effects on benzene shifts, multiplicities generated in SFORD spectra, nuclear overhauser enhancement for protonated carbons, and the comparison with analogous compounds.

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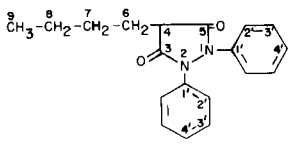
As a part of our continuous interest in assigning the carbon resonances of therapeutic agents (1), we have studied the ^{13}C nmr spectra of phenylbutazone (**1**) [4-butyl-1,2-diphenyl-3,5-pyrazolidinedione], oxyphenbutazone (**2**) [4-butyl-1-(4-hydroxyphenyl)-2-phenyl-3,5-pyrazolidinedione] and indomethacin (**3**) [1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid]. Phenylbutazone and indomethacin belong to the group of non-steroidal anti-inflammatory agents and are used in the treatment of rheumatoid arthritis. Their therapeutic effectiveness lies in between salicylate and steroidal anti-inflammatory drugs. Oxyphenbutazone is the hydroxy analogue of phenylbutazone and is one of its major active metabolites. The assignments of the carbon resonances of these anti-inflammatory drugs are of biological as well as of theoretical interest.

The ^{13}C nmr spectra of **1**, **2**, **3** and the model compound for indomethacin, indole-3-acetic acid (**4**), were obtained in DMSO-d_6 as solvent and an internal reference (39.6 ppm). In each case a proton noise-decoupled and single-frequency off-resonance decoupled (SFORD) spectra were taken. The multiplicities generated in the SFORD spectra enabled distinction between methyl, methylene, methine and quaternary carbon resonances. The assignments of the various carbon resonances were made by means of known benzene substituent effects (2), by the multiplicities generated in the SFORD spectra, by nuclear overhauser enhancement for protonated carbons, and by the comparison with structurally related compounds.



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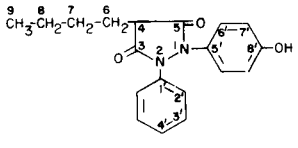

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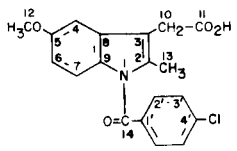

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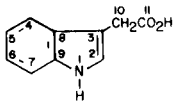

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Table I

Carbon-13 Chemical Shifts of Phenylbutazone

Assignments (a)	Multiplicity (b)	Chemical Shift (c)
C-3, C-5	s	169.9
C-1′	s	135.8
C-3′	d	128.1
C-4′	d	125.3
C-2′	d	122.3
C-4	d	45.0
C-6	t	27.0
C-7	t	26.2
C-8	t	21.4
C-9	q	12.9

(a) Numbering of carbon is shown in the structure **1**. (b) Signal multiplicity obtained from SFORD: s = singlet, d = doublet, t = triplet, q = quartet. (c) Chemical shifts are expressed in ppm relative to DMSO-d_6 (39.6 ppm).

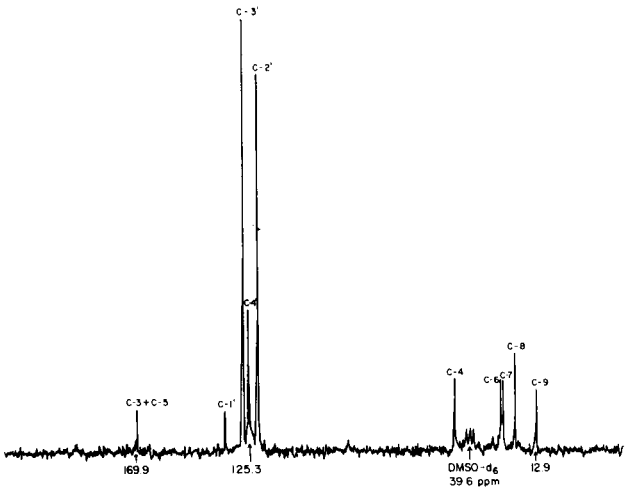


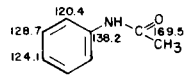
Figure 1. The proton noise decoupled ^{13}C nmr spectrum of phenylbutazone.

Phenylbutazone (1).

The ^{13}C nmr chemical shifts of phenylbutazone are recorded in Table I and illustrated in Figure I. The ten separate signals in the spectrum of **1** accounted for all the nineteen carbon resonances. The symmetrically 1,2-phenylsubstituents in phenylbutazone exhibited the ex-

pected four carbon signals in the region of 122 to 136 ppm for aromatic carbons instead of 8 signals. The five separate signals in the 12-46 ppm region have been assigned for the four carbons of the side chain and one carbon at position 4 of the pyrazolidinedione ring.

The earlier studies have reported the carbonyl resonances of amides in the region of 160 to 180 ppm (2). On this basis, the singlet at 169.9 ppm represents C-3 and C-5. The other low field singlet located at 135.8 ppm best represents the ipso carbons, C-1′, by comparing it to that of acetanilide, **5** (3).



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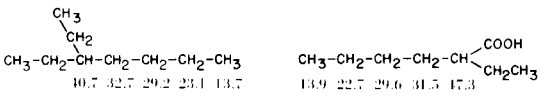
The doublets centered at 128.1, 125.3 and 122.3 ppm are assigned to C-3′, C-4′ and C-2′, respectively, by comparing them to the chemical shifts of **5** (3). Further, the electronic effect of the nitrogen atom attached to the phenyl ring produces an upfield shift of the *ortho* and *para*-carbon signals. The former appears at higher fields than the latter (2). In **1** molecule C-6 has 2α, 3β and 1γ carbons and C-7 has 2α, 2β and 2γ carbons while C-8 possesses 2α, 1β and 1γ carbons. Thus the downfield shift exhibited by α and β carbons and the upfield shift observed with γ carbons in ^{13}C nmr of **1** is in agreement with the results reported earlier (4,5). The presence of a carbonyl group in **7** (3) was reported to have no significant change on the chemical shift of C-3 as compared to the chemical shift for C-4 in **6** (6). It is, therefore, assumed that the presence of two t-amidic moieties at C-4 in **1** will not influence the chemical shift of C-6 and C-7. On these observations and the multiplicities generated in the SFORD spectrum, the chemical shifts at 45.0, 27.0, 26.2, 21.4 and 12.9 ppm in **1** are thus assigned to C-4, C-6, C-7, C-8 and C-9, respectively.



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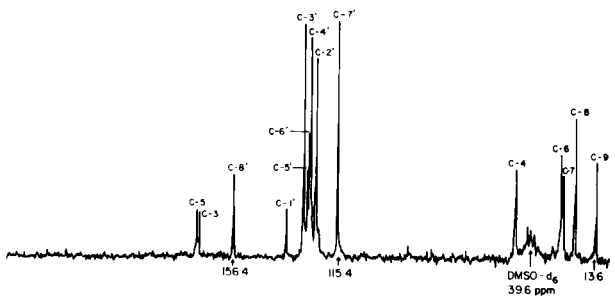


Figure 2. The proton noise decoupled ^{13}C nmr spectrum of oxyphenbutazone.

Table II

Carbon-13 Chemical Shifts of Oxyphenbutazone

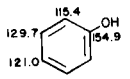
Assignments (a)	Mulitplicity (b)	Chemical Shift (c)
C-5	s	170.8
C-3	s	170.1
C-8′	s	156.4
C-1′	s	135.5
C-3′	d	128.6
C-5′	s	127.3
C-6′	d	126.0
C-4′	d	125.3
C-2′	d	122.8
C-7′	d	115.4
C-4	d	45.4
C-6	t	27.6
C-7	t	26.7
C-8	t	21.9
C-9	q	13.6

(a) Numbering of carbon is shown in the structure **2**. (b and c) See footnote in Table I.

Oxyphenbutazone (**2**).

The ^{13}C nmr spectra of **2** is represented in Figure 2 and the chemical shifts of various carbon resonances relative to dimethyl sulfoxide (DMSO-d_6) are recorded in Table II. The fifteen separate signals in the region of 13-171 ppm chemical shifts accounted for all the nineteen carbons of **2**.

The singlets at 170.8 and 170.1 ppm chemical shifts are assigned to C-5 and C-3, respectively, on the basis of earlier studies (2) and by comparing with the chemical shifts of **1** and **5** (3). The chemical shift of C-5 is represented by downfield shift in comparison to C-3 due to phenolic group. The doublets centered at 122.8, 125.3 and 128.6 ppm and one singlet at 135.5 ppm chemical shift are assigned to C-2′, C-4′, C-3′ and C-1′, respectively, by direct comparison with the chemical shifts of phenylbutazone **1** and acetanilide **5** (3). The chemical shifts for the carbons in phenol **8** (3) are in the order of *ipso* > *meta*- > *para*- > *ortho*-positions since the electronic effect of the oxygen atom proudces an upfield shift of the *ortho*- and *para*-carbon signals. The *ortho*-carbons appear at a higher field than does the *para* (2). Hence the presence of a phenolic hydroxyl group at position 8′ will shift the carbon resonances of C-5′ and C-7′ significantly upfield and C-8′ downfield in comparison to the carbon resonances of the unsubstituted phenyl ring present at position 2 of the pyrazolidinedione ring. On the basis of these observations, the singlets at 156.4 and 127.3 ppm and doublets centered at 126.4 and 115.4 ppm are assigned to the C-8′, C-5′, C-6′ and C-7′, respectively. The carbon resonances of C-4, C-6, C-7, C-8 and C-9 are assigned at 45.4, 27.6, 26.7, 21.9 and 13.6 ppm chemical shifts, respectively, by comparing these assignments with the chemical shifts of **1**, **6** (6) and **7** (3), by chemical shift



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theory (4), and by multiplicities generated in the SFORD spectra.

Indole-3-acetic Acid (**4**).

The chemical shifts of the carbon resonances of indole-3-acetic acid are reported in Table III. The triplet centered

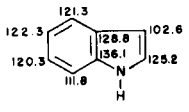
Table III

Carbon-13 Chemical Shifts of Indole-3-acetic Acid

Assignments (a)	Multiplicity (b)	Chemical Shift (c)
C-11	s	174.3
C-9	s	137.2
C-8	s	128.2
C-2	d	124.8
C-5	d	122.0
C-4, C-6	d	119.5
C-7	d	112.4
C-3	s	108.7
C-10	t	31.9

(a) Numbering of carbon is shown in the structure **4**. (b and c) See footnote in Table I.

at 31.9 ppm has been assigned for C-10. The other eight signals in the region of 108 to 175 ppm chemical shifts have been assigned for the remaining carbon resonances of **4**. The singlet at farthest lower field 174.3 ppm is attributed to C-11 on the basis of chemical shifts theory for the carbonyl carbon of carboxylic acids (2). The singlets at 137.2, 128.2 and 108.7 ppm are assigned to C-9, C-8 and C-3, respectively, by comparing the chemical shifts of indole **9** (2), by signal intensity and by substitution effects. The carbon resonances of C-2, C-5 and C-7 exhibited signals at 124.8, 122.0 and 112.4 ppm in the ^{13}C nmr spectrum of **4** which are comparable with the corresponding chemical shifts of **9**. The doublet centered at 119.5 ppm is attributed to C-4 and C-6 both on the basis of signal intensity and its comparison with the chemical shifts of **9**.



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Indomethacin (**3**).

The ^{13}C nmr chemical shifts of **3** are recorded in Table IV and illustrated in Figure 3. There are seventeen separate signals in the region of 13 to 172 ppm chemical shifts which account all the nineteen carbon resonances of **3**.

Table IV

Carbon-13 Chemical Shifts of Indomethacin

Assignments (a)	Multiplicity (b)	Chemical Shift (c)
C-11	s	172.0
C-14	s	167.7
C-5	s	155.5
C-9	s	137.6
C-2	s	135.1
C-4′	s	134.1
C-2′	d	131.0
C-1′	s	130.2
C-8	s	128.9
C-3′	d	128.3
C-3	s	114.5
C-7	d	113.4
C-6	d	111.1
C-4	d	101.8
C-12	q	55.4
C-10	t	29.6
C-13	q	13.2

(a) Numbering of carbon is shown in the structure **3**. (b and c) See footnote in Table I.

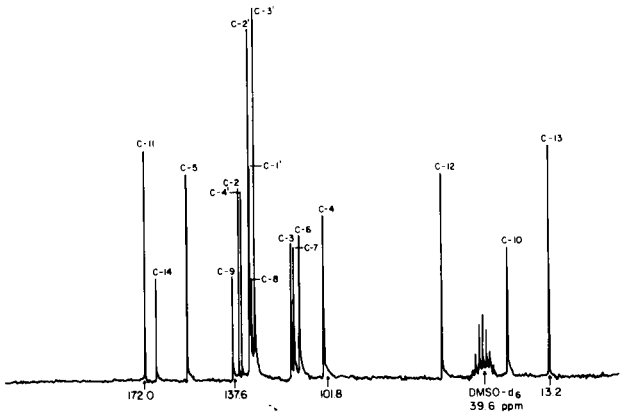
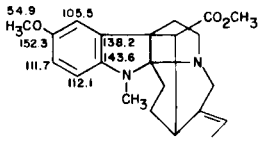


Figure 3. The proton noise decoupled ^{13}C nmr spectrum of indomethacin.

The two lower field singlets must be due to the carbon resonances of C-11 and C-14. The C-11 was differentiated from C-14 by comparing it to the chemical shift of indole-3-acetic acid. Thus, the chemical shift of C-11 and C-14 are assigned at 172.0 and 167.7 ppm, respectively. The singlet at 155.5 ppm has been assigned to C-5 since a directly bonded OCH_3 group produces a large downfield shift. This assignment is also supported by the chemical shift of the analogous carbon of **10** (7).



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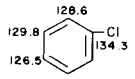
The signals exhibiting at 137.6 and 128.9 ppm are attributed to the carbon resonances of C-9 and C-8, respectively, on the basis of the direct comparison with the chemical shifts of **4** and intensity of signals. Parker and Roberts (8) reported that the substitution of a methyl group onto the 2 position of the indole nucleus causes a large downfield shift of 10.53 ppm for C-2 and an upfield shift of 2.24 ppm for C-3 in the ^{13}C nmr spectrum of 2-methyl indole in comparison to indole. Considering this observation and the chemical shifts of **4**, the singlets at 135.1 and 114.5 ppm are assigned to C-2 and C-3, respectively. Here the chemical shift of C-3 is at a lower field than expected due to the change of a secondary basic nitrogen into a tertiary amidic nitrogen. The singlet at 134.1 ppm and the doublets centered at 131.0 and 128.3 ppm have been assigned to C-4′, C-2′ and C-3′, respectively, on the basis of the signal intensity and the substitution effects of chlorine in benzene nucleus, **11** (3).



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The remaining singlet at 130.2 ppm is due to the carbon resonance of C-1′. The three doublets centered at 113.4, 111.1 and 101.8 ppm represent the resonances of C-4, C-6 and C-7. Here C-4 and C-6 are at *ortho* to methoxyl while C-7 is at the *meta* position. Earlier studies have shown that the methoxyl group produces a large upfield shift for the *ortho* and *para* carbons (2). Also C-4 is at the γ and δ psoitions to the methylene and methyl substituents, respectively, which produce an upfield shift (5). On the basis of these facts and comparing the chemical shifts of **10**, the signals at 113.4, 111.1 and 101.8 ppm are assigned to C-7, C-6 and C-4, respectively. The two quartets and one triplet centered at 55.4, 13.2 and 29.6 ppm are due to the carbon resonances of C-12, C-13 and C-10, respectively (2,5,9).

EXPERIMENTAL

The ^{13}C nmr spectra of phenylbutazone, oxyphenbutazone, indole-3-acetic acid and indomethacin were taken in DMSO-d_6 at 32° where DMSO-d_6 was used as an internal lock and a reference. The samples were run in 10 mm tube (concentration, 30% w/v). A JEOL FX 60 spectrometer, operating at 15.00 KHZ, was used and pulse Fourier transform technique was applied. The spectrometer setting during experiment was as following: repetition 2.5 sec and data point 4K. All chemicla shifts were calculated from the digitized spectra by the computer.

In the present study phenylbutazone, oxyphenbutazone and indomethacin were obtained from CIBA Pharmaceutical Company Division of CIBA-GEIGY Corporation, Summit, N. J., and indole-3-acetic acid was purchased from Sigma Chem. Company, St. Louis, Missouri. All compounds were recrystallized from suitable solvents before taking their ^{13}C nmr spectra.

Acknowlegement.

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