# Studies on Aloe. Part 6.[1] Conformation and Absolute Configuration of Aloins A and B and Related 10-*C*-Glucosyl-9-anthrones†

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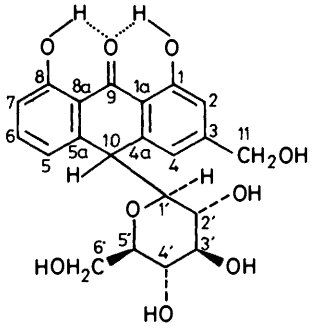
The conformation and absolute configuration of the two diastereoisomeric aloins A (**2a**) and B (**2b**) were proven on the basis of shielding and deshielding effects and specific NOE (nuclear Overhauser effect) associations in $^1$H NMR spectroscopy. Previous $^1$H NMR assignments were corrected and $^{13}$C NMR spectra recorded for the first time. Taking into account analogies in CD spectra as well as in NOE effects, the absolute configuration of C-10 was suggested for the epimeric pairs of naturally occurring 1,8-dihydroxy-10-*C*-β-D-glucopyranosyl-9-anthrones.

Aloins A and B are two diastereoisomeric *C*-glucosides (**1**) differing in the configuration of C-10 in the aloe-emodin anthrone moiety.[2–6] They are collectively called barbaloin[7] in that their unresolved mixture was first isolated from Barbados aloe.[8] The constitution of barbaloin was extensively investigated during the first half of this century,[9] definitely clarified in 1956,[10] and confirmed by synthesis in 1980.[3] The β-configuration of the glucosyl residue was inferred by synthesis[3] and proven by $^1$H NMR spectroscopic evidence.[6]




(**1**)

It is generally accepted that barbaloin represents the bitter and purgative principle of the drug aloe (or aloes) which is produced by hot drying of the leaf exudate of a number of *Aloe* species.[11] Cape aloe, the currently commonly available aloe of commerce, is prepared from *A. ferox* Mill. and contains 9–16% barbaloin.[7] This has recently been shown to occur in 85 out of the 240 *Aloe* species so far investigated.[12] It has also been found in extracts from cascara bark (*Rhamnus purshiana* D.C.).[13]

The two aloins rapidly interconvert in the presence of bases[2,4] and are distinguishable only by reverse-phase high-performance liquid chromatography (HPLC)[2,4] or droplet counter-current chromatography.[5] In TLC they move as one spot, staining green when sprayed with the diazo-reagent Fast Blue B.[12] For this reason the two aloins are conventionally individualized by their retention times in HPLC [LiChrosorb RP-8 or RP-18, acetonitrile–water (25:75, v/v)], aloin A being the less polar and slower moving compound.[3] Aloin A may also be obtained by crystallization of the crude mixture from methanol.[3]

Biosynthetic studies in *A. arborescens* Mill. revealed that aloin B is actively synthesized by the plant and transformed in part into aloin A.[14]

Although Auterhoff *et al.*[3] reported a configuration assignment to C-10 in each epimeric aloin, the problem must be considered still open. In fact, these authors based their conclusions on misinterpreted $^1$H NMR spectra of aloins. Their reasoning also appeared theoretically inconsistent. In particular the signal of the hydroxy proton in the 2′-position of the sugar moiety was assigned to the anomeric proton erroneously, as pointed by Rauwald and Roth.[6] This prompted us to reinvestigate the absolute configuration of aloins A and B.

## Results

*Nuclear Magnetic Resonance Spectra and Nuclear Overhauser Enhancement Experiments.*—$^1$H NMR assignments for both aloins in two different solvents were obtained with the aid of extensive homonuclear decoupling experiments and one-bond heteronuclear 2D correlations (Table 1). The use of the mixture DMSO–$CDCl_3$ (1:3, v/v) as a solvent for recording NMR spectra appeared to be convenient to reduce the aloin A–aloin B interconversion, which cannot be neglected in long-term experiments in DMSO, as well as to obtain a better resolution of aromatic protons.

Spectral data (in DMSO) were found in good agreement with those previously reported by Rauwald and Roth,[6] except for 5-H and 7-H, which had to be interchanged. The unambiguous assignments for these protons rest on the following nuclear Overhauser effect (NOE) experiments: in aloin A 4% intensity enhancement was observed for the 8-hydroxy proton by irradiation of 7-H in DMSO (and 3.4% for 1-OH from 2-H); in addition, irradiation of the anomeric proton in DMSO–$CDCl_3$ (1:3, v/v) resulted in an enhancement (4.2%) of the signal of 5-H. Analogous results were obtained for aloin B. However, it must be noted that in this diastereoisomer the signal of 4-H (not 5-H) was found to be enhanced (5.8%) by irradiation of 1′-H.

A comparison of the chemical shifts of the carbohydrate residue in aloins A and B with those of β-D-glucopyranose in DMSO[15] showed that the resonances of protons at the 4′-, 5′-, and 6′-position and of the 6′-hydroxy group are shifted upfield on going from glucose to aloins ($\Delta\delta$ *ca.* −0.30, −0.35, −0.30, and −0.60 ppm, respectively), whereas a marked downfield shift occurs for the 2′-OH signal ($\Delta\delta$ *ca.* +0.40 ppm). Finally, it must be noted that the coupling constant between 10-H and 1′-H is small and identical for aloins A and B.

† This Paper is also Part 2 of the series 'Conformational Studies of Natural Products;' for the Part 1, see D. Monti, P. Manitto, S. Tagliapietra, G. Dadà, and G. Speranza, *Gazz. Chim. Ital.*, 1986, **116**, 303.

**Table 1.** $^1$H NMR (300 MHz) peaks of aloins A and B at 25 °C. Splitting patterns and *J*-values (Hz) are given in parentheses[a].

| Proton | Aloin A | | Aloin B | |
|---|---|---|---|---|
| | DMSO | DMSO–$CDCl_3$ (1:3) | DMSO | DMSO–$CDCl_3$ (1:3) |
| 2-H | 6.86 (s) | 6.80 (s) | 6.82 (s) | 6.78 (s) |
| 4-H | 7.04(s) | 7.00 (s) | 7.01 (s) | 6.95 (s) |
| 5-H | 7.08 (d, 8.0) | 6.94 (d, 8.0) | 7.08 (d, 8.0) | 7.01 (d, 8.0) |
| 6-H | 7.57 (dd, 8.0) | 7.39 (dd, 8.0) | 7.54 (dd, 8.0) | 7.36 (dd, 8.0) |
| 7-H | 6.89 (d, 8.0) | 6.77 (d, 8.0) | 6.89 (d, 8.0) | 6.77 (d, 8.0) |
| 10-H | 4.57 (d, 2.0) | 4.54 (d, 2.0) | 4.57 (d, 2.0) | 4.54 (d, 2.0) |
| 11-$H_2$ | 4.56 (d, 6.0) | 4.54 (d, 6.0) | 4.56 (d, 6.0) | 4.54 (d, 6.0) |
| 1′-H | 3.28 (dd, 9.5, 2.0) | 3.29 (dd, 9.5, 2.0) | 3.30 (dd, 9.5, 2.0) | 3.30 (dd, 9.5, 2.0) |
| 2′-H | 2.79 (dd, 9.5) | 2.85 (dd, 9.5) | 2.77 (dd, 9.5) | 2.93 (dd, 9.5) |
| 3′-H | 3.08 (dd, 9.5) | 3.19 (dd, 9.5) | 3.07 (dd, 9.5) | 3.22 (dd, 9.5) |
| 4′-H | 2.63–2.80 (m) | 2.75–2.85 (m) | 2.60–2.75 (m) | 2.75–2.90 (m) |
| 5′-H | 2.63–2.80 (m) | 2.75–2.85 (m) | 2.60–2.75 (m) | 2.75–2.90 (m) |
| 6′-$H_a$ | 3.16 (dd, 11.0, 5.0) | 3.29 (dd, 11.0, 5.0) | 3.08 (dd, 11.0, 5.0) | 3.27 (dd, 11.0, 5.0) |
| 6′-$H_b$ | 3.38 (dd, 11.0, 1.8) | 3.41 (dd, 11.0, 2.0) | 3.34 (dd, 11.0, 1.8) | 3.39 (dd, 11.0, 2.0) |
| 1-OH | 11.81 (s) | 11.76 (s) | 11.78 (s) | 11.77 (s) |
| 8-OH | 11.90 (s) | 11.85 (s) | 11.84 (s) | 11.82 (s) |
| 11-OH | 5.44 (t, 6.0) | | 5.42 (t, 6.0) | |
| 2′-OH | 5.15 (d, 6.0) | | 5.28 (d, 6.0) | |
| 3′-OH | 4.91 (d, 4.5) | | 4.91 (d, 5.0) | |
| 4′-OH | 4.76 (d, 5.0) | | 4.74 (d, 5.0) | |
| 6′-OH | 3.94 (t, 5.5) | | 3.85 (t, 5.5) | |

[a] Spectral simplification was induced by exchange of the hydroxy protons with $D_2O$, except for the estimate of hydroxy proton coupling constants.

**Table 2.** $^{13}$C NMR (75.47 MHz) peaks (ppm) of aloins A and B at 25 °C.

| Carbon | Aloin A | | Aloin B | |
|---|---|---|---|---|
| | DMSO | DMSO–$CDCl_3$ (1:3) | DMSO | DMSO–$CDCl_3$ (1:3) |
| 1 | 160.8 | 161.0 | 160.9 | 161.2 |
| 2 | 112.7 | 112.7 | 112.4 | 112.5 |
| 3 | 151.4 | 150.8 | 152.2 | 151.6 |
| 4 | 117.8 | 117.7 | 120.3 | 119.9 |
| 5 | 118.9 | 118.4 | 116.3 | 116.1 |
| 6 | 136.1 | 135.5 | 135.3 | 134.7 |
| 7 | 115.4 | 115.3 | 115.8 | 115.6 |
| 8 | 161.1 | 161.4 | 160.9 | 161.2 |
| 9 | 193.4 | 193.7 | 193.4 | 193.7 |
| 10 | 44.2 | 44.1 | 43.9 | 44.0 |
| 1a | 115.8 | 115.8 | 115.7 | 115.7 |
| 4a | 142.0 | 141.4 | 141.8 | 141.4 |
| 5a | 145.6 | 145.3 | 145.8 | 145.3 |
| 8a | 117.1 | 117.0 | 117.4 | 117.3 |
| 11 | 62.5 | 62.9 | 62.4 | 62.9 |
| 1′ | 85.2 | 85.6 | 85.1 | 85.5 |
| 2′ | 70.3 | 70.0 | 70.1 | 70.0 |
| 3′ | 78.2 | 78.6 | 78.2 | 78.6 |
| 4′ | 70.3 | 70.6 | 70.3 | 70.6 |
| 5′ | 80.9 | 79.6 | 80.8 | 79.6 |
| 6′ | 61.4 | 62.0 | 61.4 | 62.0 |

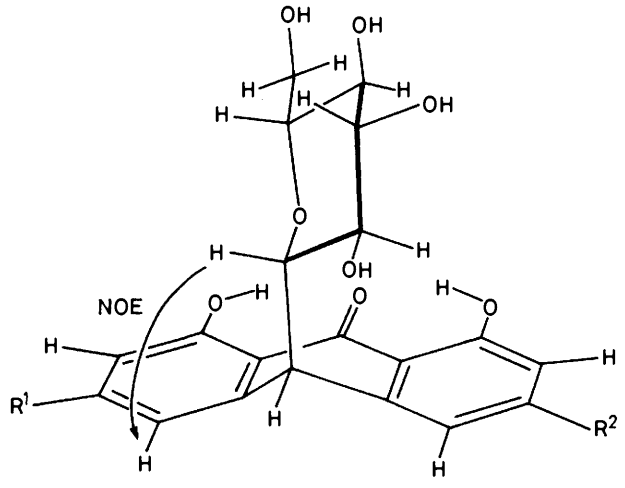
*$^{13}$C Nuclear Magnetic Resonance Spectra and 2D Correlations.*—$^{13}$C NMR resonances of aloins are reported for the first time in Table 2. Off-resonance and DEPT spectra allowed 2 methylene, 11 methine, and 8 quaternary carbon atoms to be distinguished. Assignments were mainly based on analogies of chemical shifts with those of anthrones[16,17] and 1,8-dihydroxy-9,10-anthraquinones[18] as well as on $^1$H–$^{13}$C 2D correlations. Long-range 2D heterocorrelated experiments, when applied to aloin A, revealed two- and three-bond connectivities and confirmed the chemical-shift assignments of all quaternary carbons. The $^{13}$C signal at δ 161.4 exhibited long-range correlations with proton resonances at δ 7.39 (6-H) and 11.85 (8-OH), thus allowing the carbon signal to be assigned to C-8. The complementary connectivity (1-OH)–(C-1)–(2-H) was also shown. Analogously, evidence was obtained for the connections of C-8a with 7-H and 5-H, of C-1a with 2-H and 4-H, and of C-3 with 11-$H_2$. The $^{13}$C-peaks at δ 141.4 and at 145.3 were found to be correlated with the 10-H (δ 4.54) and were assigned to C-4a and 5a, respectively, the latter carbon being also long-range-coupled to 6-H.

Chemical shifts for the carbohydrate carbons of both aloins follow the usual pattern for β-D-glucopyranose,[19] except for the C-1′ resonance which is at higher field than those found in O-β-D-glucosides[19] and at lower field compared with *C*($sp^2$)-β-D-glucosides.[20,21]

The larger low-field shift at C-9 in aloin with respect to the unsubstituted anthrone[16] and anthraquinone[18] (Δδ *ca.* 10 ppm) is presumably due to the enhancement of the polarization of the C=O bond by the two *peri*-OH groups. X-Ray analysis of 1,8-dihydroxy-9-anthrone (anthralin)[22] showed that the molecule is almost planar and includes intramolecular O–H ··· O ··· H–O bonds associated with an elongated C=O length of 1.261 Å.

## Discussion

An interesting feature of the $^1$H NMR spectra of both aloins is the distinctive upfield shifts observed for 4′-, 5′-, 6′-H, 6′-OH, and the downfield shift for 2′-OH. These strong differences in chemical shift with respect to the corresponding protons of the β-D-glucopyranose are best explained in terms of shielding and deshielding effects of the anthrone moiety. Taking into account the value of the H(10)–H(1′) coupling constant (2.0 Hz in both stereoisomers), the structure (2), having a calculated[23] torsional angle H–C(10)–C(1′)–H of *ca.* −57°, appears to be the most likely candidate for the preferred conformation of aloins A and B in solution. This conformation, having the D-glucopyranosyl group in a quasi-axial position, is in agreement with what is




(2)

**a**; $R^1$ = H, $R^2$ = $CH_2OH$

**b**; $R^1$ = $CH_2OH$, $R^2$ = H

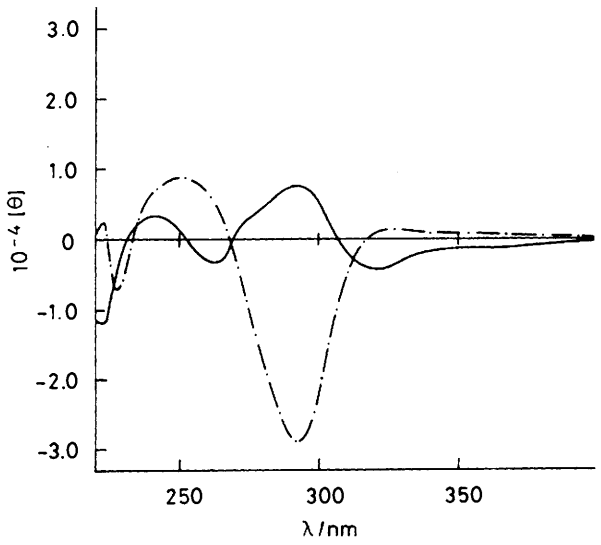



**Figure.** CD spectra of aloin A (**2a**) (——) and aloin B (**2b**) (— · — · —) in MeOH.

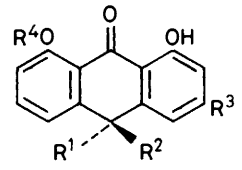
known about the conformational stability of 10-substituted 9-anthrones.[24–28] In these molecules the central ring of the anthracenyl system adopts a 'flattened boat' conformation with the large substituent axial to avoid steric interactions with the *peri*-hydrogen atoms at C-4 and C-5.[27] Inspection of molecular models justifies the marked magnetic shifts, as reported for groups in the 10-position of 9-anthrone.[24–28]

In addition, it can be pointed out that MM2 calculations,* performed on compound (**2**) ($R^1$ = $R^2$ = H), using bond distances reported in the literature for 1,8-dihydroxy-9-anthrone[22] and β-D-glucose,[29] and a standard value (1.54 Å) for the C(10)–C(1′) distance, indicated the existence of one energy minimum for the rotation about the C(10)–C(1′) bond corresponding to a torsional angle H–C–C–H of $-56 \pm 2°$.

Assuming that the pyranosyl residue spends the greater part of its time over the plane of the anthracenyl moiety with the ring oxygen close up to the carbonyl group, the different NOEs observed for aloins A and B can be used to determine the C-10 configuration in each epimer. In fact, the NOE associations of 1′-H with *5-H in aloin A* and with *4-H in aloin B* were shown to be consistent with the preferred conformation (**2a**) for the former stereoisomer and (**2b**) for the latter. Thus, aloin A is (10*S*)-barbaloin (**3**) and aloin B its 10-epimer (**4**).

It is noteworthy that the CD spectra of the two aloins clearly reflect the opposite configuration of C-10 (Figure). Hence the alternate sign of the Cotton effects in the ranges 270–310, and 310–340 nm (or the sequence of maxima/minima along the spectrum), and the specific NOE association of the anomeric proton with one hydrogen atom in a *peri*-position appear to be of diagnostic value in determining the absolute configuration of 10-*C*-β-D-glucopyranosyl-9-anthrones asymmetrically substituted in the anthrone moiety.

On this basis, using spectroscopic data reported in the literature, it has been possible to assign the C-10 configuration to epimeric pairs of aloin-like compounds. These can be divided in two groups, each one being characterized by the chirality sense of the helical array C(4a)–C(10)–C(1′)–O [where C-4a is the angular carbon of the C-3-substituted ring in




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| (**3**) | H | β-D-Glc*p* | $CH_2OH$ | H |
| (**4**) | β-D-Glc*p* | H | $CH_2OH$ | H |
| (**5**) | OH | β-D-Glc*p* | $CO_2H$ | β-D-Glc*p* |
| (**6**) | β-D-Glc*p* | OH | $CO_2H$ | β-D-Glc*p* |
| (**7**) | H | β-D-Glc*p* | $CO_2H$ | β-D-Glc*p* |
| (**8**) | β-D-Glc*p* | H | $CO_2H$ | β-D-Glc*p* |
| (**9**) | H | β-D-Glc*p* | $CH_2OH$ | β-D-Glc*p* |
| (**10**) | β-D-Glc*p* | H | CH OH | β-D-Glc*p* |

the 'locked' conformation (**2**)]. The *P*-helicity group[30] includes, besides aloin A (**3**), rheinoside B (**5**) (10*R*), rheinoside D (**7**) (10*S*), and cascaroside A (**9**) (10*S*). The *M*-helicity group[30] includes, besides aloin B (**4**), rheinoside A (**6**) (10*S*), rheinoside C (**8**) (10*R*), and cascaroside B (**10**) (10*R*). The structures of rheinosides rest on CD spectra and NOE associations,[31] whereas those of cascarosides rest on CD spectra only.[32]

## Experimental

$^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker CXP 300 in $(CD_3)_2SO$ and $(CD_3)_2SO$–$CDCl_3$ (1:3, v/v), using the $(CD_3)_2SO$ signal as internal standard (2.50 and 39.50 ppm from $SiMe_4$ for $^1H$ and $^{13}C$, respectively). NOE experiments were performed by using the 'N.O.e Difference' microprogram included in the Aspect 2000 computer library. CD spectra were obtained on a Jasco-500 instrument. Analytical and preparative HPLC was performed on a Perkin-Elmer Series 3B liquid chromatograph connected to a variable-wavelength UV detector (Perkin-Elmer LC 85 Spectrophotometric Detector).

*Aloin A* (**2a**).—A commercial sample of aloin (Merck) was repeatedly crystallized from MeOH–$CHCl_3$ (1:4. v/v) to give pure compound (**2a**).[3,5]

* MM2 1977 (Allinger-QCPE 395) + MMP1 Pi (Allinger-QCPE 318) + MODEL PRMTRS (Still) + MORE ATOMS & TRIAL CONSTANTS, by K. E. Gilbert and J. J. Gajewski.

*Aloin B* (**2b**).—A solution of aloin A (400 mg) in DMSO (5 ml) was heated at 100 °C for 10 min. After removal of the solvent, the residue was shown to contain the two isomers (**2a**) and (**2b**) in the ratio 1:1 by analytical HPLC [column, LiChrosorb RP-18 (10μ, 4 mm id × 25 cm); mobile phase, MeOH–water (45:55, v/v); flow rate, 2.0 ml $min^{-1}$; detector, UV (360 nm); $t_R$ 7.2 min]. Isolation of aloin B was achieved by preparative HPLC [column, LiChrosorb RP-18 (7μ, 10 mm id × 25 cm); mobile phase, MeOH–water (45:55, v/v); flow rate, 8 ml $min^{-1}$; detector, UV (300 nm); $t_R$ 15.8 min]. Eluates of the column were collected, concentrated under reduced pressure, and lyophilized to give compound (**2b**) (140 mg, 35%)[3,5] which was shown to be pure by analytical HPLC.

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