Studies on Aloe. 15.[1] Two New 5-Methylchromones from Cape Aloe

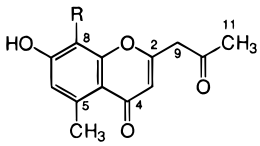
Giovanna Speranza,* Gabriele Fontana, Simona Zanzola, and Antonella Di Meo

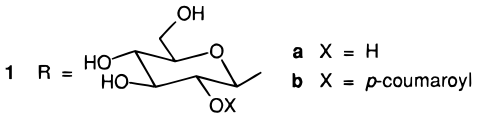
Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, via Venezian 21, 20133 Milano, Italy

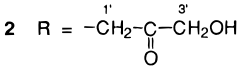
Received June 24, 1996[⊗]

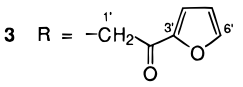
Further investigation of Cape aloe led to the isolation of two new 5-methylchromones. Their structures have been established as 2-acetonyl-7-hydroxy-8-(3-hydroxyacetonyl)-5-methylchromone (**2**) and 2-acetonyl-8-(2-furoylmethyl)-7-hydroxy-5-methylchromone (**3**) on the basis of spectroscopic data. A mechanistic hypothesis to account for their occurrence in the drug is presented.

5-Methylchromones bearing a *C*-glucosyl residue at 8-position are typical metabolites of *Aloe* spp.[2,3] Thus, aloesin (**1a**) and aloeresin A (**1b**) are the most abundant components of Cape aloe, the dried latex from the cut leaves of *Aloe ferox* Mill.[4] As a part of our continuing investigations on Cape aloe, aimed at the complete chemical characterization of the drug, we isolated two new nonglucosylated 8-substituted 5-methylchromones, compounds CA-15 and CA-16. Their isolation and structure elucidation as **2** and **3**, respectively, are reported here.









Compounds **2** and **3** were isolated from an Me_2CO−$CHCl_3$ extract of powdered Cape aloe by a combination of chromatographic techniques. During the fractionation of the extract, they appeared as orange spots when a 0.5% aqueous solution of Fast Blue B salt was used as TLC detection reagent, thus suggesting a close structural relationship to aloesin (**1a**) and aloeresin A (**1b**).[5] In addition, the EIMS spectra of both compounds exhibited intense peaks at *m*/*z* 245, 203, and 163, which were familiar from *C*-glucosylated 2-acetonyl-5-methylchromones.[6,7] ^{1}H- and ^{13}C-NMR spectra (Table 1) also showed resonances assignable to a 2-acetonyl-5-methylchromone unit, in particular, the signal at δ 2.66 (aromatic methyl group at 5-position), the sharp singlet at δ 6.05 (H-3 of the pyrone ring), and the signals of the easily D_2O-exchangeable CH_2 group of the acetonyl side chain (δ_H 3.78, δ_C 47.12 for **2** and δ_H 3.70, δ_C 47.16 for **3**).[2]

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds CA-15 (**2**) and CA-16 (**3**) in DMSO-d_6 at 25 °C[a]

	2		**3**	
position	δ_H[b]	δ_C	δ_H[b]	δ_C
1a		157.07		157.17
2		160.67		160.28
3	6.05 (s)	112.73	6.05 (s)	112.52
4		178.93		178.42
4a		114.58		114.25
5		139.60		139.35
6	6.69 (s)	115.70	6.70 s)	115.61
7		159.11		159.02
8		107.48		107.11
9	3.78 (s)	47.12	3.70 (s)	47.16
10		203.25		202.26
11	2.19 (s)	29.98	2.07 (s)	29.63
CH_3-Ar	2.66 (s)	22.62	2.66 (s)	22.45
1′	3.80 (s)	32.84	4.21	32.62
2′		207.96		185.04
3′	4.15 (s)	67.45		151.61
4′			7.54 (d, 3.5)	118.46
5′			6.74 (dd, 3.5, 1.7)	118.46
6′			8.01 (d, 1.7)	112.52
7-OH	10.50 (br s)		10.52 (br s)	
3-OH	5.15 (br s)			

[a] Signal assignments were based on DEPT, HETCOR, and COLOC experiments and on analogies of chemical shifts with those found for the chromone derivatives previously isolated from Cape aloe (see Speranza *et al.*[2]). [b] Splitting patterns and *J* values (Hz) are given in parentheses.

For compound **2**, the remaining signals in the ^{13}C- and DEPT NMR spectra at δ 32.84, 67.45, and 207.96 were consistent with a methylene, a hydroxymethylene, and a carbonyl group. The multiplicity of their respective protons in the ^{1}H-NMR spectrum (singlets at δ 3.80 and 4.15 for CH_2 and CH_2OH, respectively, as deduced by a HETCOR experiment) was in agreement with the presence of a CH_2COCH_2OH grouping. This could be placed at C-8 on the basis of NOE experiments, in that irradiation of the 5-Me resulted in the enhancement of the H-6 signal and vice versa.

These data, together with the molecular formula $C_{16}H_{16}O_6$, as determined by HRMS (*m*/*z* 304.0966, calcd 304.0946), clearly indicated the structure 2-acetonyl-7-hydroxy-8-(3-hydroxyacetonyl)-5-methylchromone (**2**) for compound CA-15.

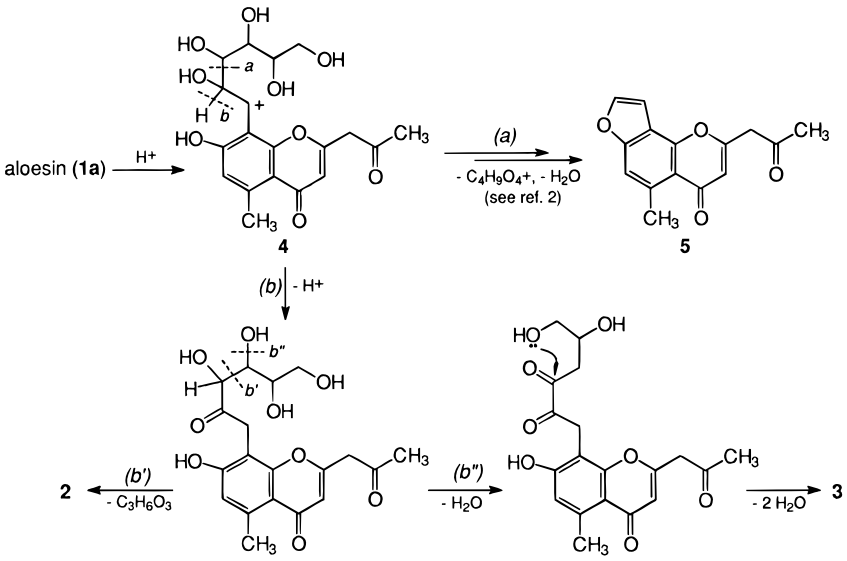
The molecular formula of compound CA-16 (**3**) was found to be $C_{19}H_{16}O_6$ by HREIMS analysis (found, *m*/*z* 340.0904, calcd 340.0946). The ^{1}H-NMR spectrum, besides signals caused by the chromone moiety, dis-

* To whom correspondence should be addressed. Phone: +39-2-236 75 98. FAX: +39-2-236 43 69. E-mail: giospe@icil64.cilea.it.

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1997.

S0163-3864(96)00531-9 CCC: $14.00

Scheme 1



played a two-proton singlet at δ 4.21 and two one-proton doublets at δ 7.54 (J = 3.5) and 8.01 (J = 1.7) coupled with the one-proton double doublet at δ 6.74 (J = 3.5, 1.7 Hz), suggesting the presence of an isolated methylene group and an α-substituted furan ring.[8] These structural features were confirmed by ^{13}C-NMR data (see Table 1), which also revealed the existence of an additional carbonyl group at δ 185.04.

These findings are indicative of a 5-methylchromone nucleus connected to a furan moiety through a $-CH_2-CO-$ bridge. The NOE association observed between the 5-methyl group and H-6 excluded the attachment of the bridge at C-6 of the chromone unit. That the methylene was linked to C-8 and the carbonyl group to the α-position of the furan ring was then inferred by these observations: (a) the ^{13}C signal of the carbonyl group falls at ca. 10 ppm upfield compared to alkyl phenyl ketones, but its value is characteristic of alkyl furyl ketones;[9] and (b) a fragment peak at m/z 95, diagnostic of a furoyl group,[10] is present in the EIMS spectrum. Therefore, the structure 2-acetonyl-8-(2-furoylmethyl)-7-hydroxy-5-methylchromone (**3**) was assigned to compound CA-16.

In regard to the origin of compounds **2** and **3**, the hypothetical routes outlined in Scheme 1 appear to be mechanistically plausible. It seems reasonable to assume the intermediacy of a common, stabilized benzylic cation (**4**) (or an equivalent) in the formation of **2** and **3** as well as of the previously described compound **5**.[2] Thus, all three constituents of Cape aloe would arise from an acid-catalyzed degradation of the *C*-glucosyl residue of aloesin (**1a**).

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H- (300.13 MHz) and ^{13}C-NMR (75.47 MHz) were recorded on a Bruker AC 300 spectrometer equipped with an ASPECT 3000 data system, using the solvent signal as an internal standard (DMSO-d_6: δ_H 2.50, δ_C 39.50). MPLC separations were carried out on a Büchi borosilicate glass column (46 cm × 36 mm i.d.) with a Perkin-Elmer HPLC pump (Series 3B) and a Waters 484 UV detector at 254 nm. A Waters Model 600 E liquid chromatograph connected to a HP 1050 diode array detector was used for analytical HPLC. For IR and MS instruments, as well as for general experimental procedures for TLC and flash chromatography, see Speranza *et al.*[1,3]

Drug Sample. The commercial Cape aloe used in this investigation was purchased from D. Ulrich Spa (Nichelino, Italy) and was produced in the Port Elisabeth region of Cape Town, South Africa. A voucher specimen is on deposit in the Dipartimento di Chimica Organica e Industriale, University of Milan.

Extraction and Isolation of Compounds. The finely powdered drug (4.0 kg) was extracted as previously described.[3] The brown syrup, obtained after treatment with hexane, was adsorbed on sea sand (100 g) with constant stirring until completely dried. It was fractioned by flash chromatography (Si gel, 1.0 kg) eluting with EtOAc−EtOH−H_2O (100:20:13). After TLC analysis (eluent as above, spray reagent: Fast Blue B salt, 0.5% in H_2O[3]), fractions containing compounds CA-15 (R_f 0.51) and CA-16 (R_f 0.62) were combined and evaporated under reduced pressure to give 5.7 g of residue. This was subjected in two aliquots to MPLC on RP-18 Si gel (LiChroprep, Merck, 25−40 μm) with MeOH−H_2O (7:3) as eluting solvent. The flow rate was 5 mL/min, and 10-mL fractions were collected. Fractions 10−18, containing compound CA-15, were evaporated, and the residue (380 mg) was further purified by flash chromatography on Si gel using $CHCl_3$−EtOAc−MeOH (100:3:1) as eluent. After recrystallization from MeOH, compound CA-15 (**2**) (104 mg, 0.003% yield) was obtained as fine needles, pure by TLC and analytical HPLC (column: LiChrospher100 RP-18, 5μm, 125 × 4 mm, Merck; flow rate, 1 mL/min; UV detection at λ 254 nm; mobile phase, MeOH−H_2O, linear gradient from 10 to 60% MeOH in 25 min; t_R 16.5 min); mp 208−210 °C; IR (KBr) ν_{max} 3423, 1718, 1654, 1610, 1596, 1577, 1507, 1458, 1389 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; NOE associations (% intensity enhancement), from H-6 to 5-Me (3.8), and from 5-Me to H-6 (12.3); EIMS (70 eV) m/z (rel int) 304 $[M]^+$ (63), 245 (100), 203 (27), 163 (33).

The residue obtained from fractions 20−31 after evaporation of the solvent, was recrystallized from

MeOH giving 43 mg (0.001% yield) of compound CA-16 (**3**) pure by TLC and analytical HPLC (t_R 20.1 min, chromatographic conditions as above): mp 246−248 °C; IR (KBr) ν_{max} 3438, 1717, 1656, 1603, 1577, 1506, 1467, 1384 cm^{-1}; ^1H- and ^{13}C-NMR data, see Table 1; NOE associations (% intensity enhancement), from H-6 to 5-Me (4.2) and from 5-Me to H-6 (13.4); EIMS (70 eV) *m/z* (rel int) 340 $[M]^+$ (62), 245 (100), 203 (20), 163 (32), 95 (63).

Acknowledgment. We are grateful to Prof. P. Manitto, University of Milan, for helpful discussion and to Dr. D. Monti, CNR, Italy, for running NMR spectra. Thanks are due to MURST of Italy for financial support.

References and Notes

(1) Part 14: Speranza, G.; Di Meo, A.; Manitto, P.; Monti, D.; Fontana, G. *J. Agric. Food Chem.* **1996**, *44*, 274−277.
(2) Speranza, G.; Manitto, P.; Cassarà, P.; Monti, D.; De Castri, D.; Chialva, F. *J. Nat. Prod.* **1993**, *56*, 1089−1094, and references cited therein.
(3) Speranza, G.; Corti, S.; Manitto, P. *J. Agric. Food Chem.* **1994**, *42*, 2002−2006, and references cited therein.
(4) Tyler, V. E.; Brady, L. R.; Robbers, J. *Pharmacognosy*; Lea and Febiger: Philadelphia, 1988; pp 62−63.
(5) Reynolds, T. *Bot. J. Linn. Soc.* **1985**, *90*, 179−199.
(6) Speranza, G.; Gramatica, P.; Dadà, G.; Manitto, P. *Phytochemistry* **1985**, *24*, 1571−1573.
(7) Such fragments can be interpreted as arising from the loss of the glucosyl residue with retention of a CH_2^+ group (*m/z* 245) and from further fragmentations (*m/z* 203, 163) of this chromone cation; cf.: Markham, K. R.; Mabry, T. J. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, H., Eds.; Chapman & Hall: London, 1975; pp 118−121.
(8) Fringuelli, F.; Gronowitz, S.; Hörnfeldt, A. B.; Johnson, I.; Taticchi, A. *Acta Chem. Scand. B* **1974**, *28*, 175−184.
(9) Hearn, M. T. W. *Aust. J. Chem.* **1976**, *29*, 107−113.
(10) Bohlmann, F.; Zdero, C. *Phytochemistry* **1978**, *17*, 1155−1159.

NP960531U