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A method for detection of transesterification in solutions of esters of quinoline-3-carboxylic acids

A number of compounds possessing the 4-hydroxyquinoline-3-carboxylic acid structure are of importance as coccidiostats and antibacterial agents. For example, ethyl-4-hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate (buquinolate)[1], methyl 7-benzyloxy-6-butyl-1,4-dihydro-4-oxo-3-quinolinecarboxylate[2] and, ethyl 6-(decyloxy)-7-ethoxy-4-hydroxy-3-quinolinecarboxylate (decoquinate)[3,4] have been shown to exhibit prophylactic activity against various species of coccidia in broiler chickens[5–8].

While developing chromatographic methods for study of decoquinate it was found that a second chromatographic component was formed in methanolic solutions of the compound. The unknown was isolated and identified as the methyl ester analogue of decoquinate. The present report describes thin-layer chromatographic (TLC) methods to detect this transesterification which esters of quinoline-3-carboxylic acids undergo easily in alcoholic solvents. Thus the technique separates closely related methyl and ethyl esters.

Experimental

Materials. Decoquinate or ethyl 6-(decyloxy)-7-ethoxy-4-hydroxy-3-quinolinecarboxylate was obtained from May & Baker Ltd., Great Britain. The methyl ester analogue of decoquinate was synthesized in our laboratory by methanolysis of ethyl 6-(decyloxy)-7-ethoxy-4-hydroxy-3-quinolinecarboxylate. Silica Gel G was supplied by Brinkmann Instruments Inc., Westbury, N. Y.; "wick sticks" (pressed KBr sticks) by Harshaw Chemical Co., Cleveland, Ohio. Other chemicals used were reagent and spectrophotometric grade quality.

Analytical TLC. Thin-layer plates (2 × 8 in.) were prepared by spreading a slurry of 30 g of Silica Gel G with 70 ml of distilled water on glass plates with a Desaga applicator adjusted for 0.20 mm layer thickness. The plates were air-dried for 30 min at room temperature, then oven-dried for 2 h at 110°.

Samples containing 40–80 μg of decoquinate were applied with a micropipet as a zone 2 cm from the bottom edge of the plate, care being taken to evaporate the solvent in an air stream. The chromatoplates were developed by ascending technique in a rectangular chamber with toluene–absolute ethanol–glacial acetic acid (5:1:1). The plates were dried in an oven at 60° and the positions of fluorescent zones determined under UV light.

The relative amount of each zone was measured fluorometrically[9]. Each zone was scraped from the plate and extracted with 25 ml of 1 % $CaCl_2$ in methanol. The fluorescence of the solution was determined using an activation wavelength at 325 mμ and emission filter at 390 mμ employing a Farrand model A filter fluorometer.

Preparative TLC. Preparative plates (4 × 8 in.) were made in the same manner as the analytical plates except that the Desaga spreader was adjusted for 0.5 mm thickness. Plates were air-dried for 60 min at room temperature and 2 h at 110°. Approximately 200–1000 μg of decoquinate were applied as a zone 2 cm from the bottom of the plate. The chromatoplates were developed by ascending technique with toluene–absolute ethanol–glacial acetic acid (5:1:1). After 4 h, the plates were re-

moved from the chamber and oven-dried at 60°. The position of fluorescent zones was determined under UV light. Isolation of fluorescent components was achieved by scraping the zones from the TLC plate into a 50 ml erlenmeyer flask. Approximately 25–30 ml of chloroform–methanol solvent (1:1) was added, the mixture shaken on a wrist shaker for 15 min and filtered through a medium pore glass filter into a 100 ml round bottom flask. The solution was evaporated to dryness and redissolved in 3 ml methanol. Precipitation was achieved by the addition of distilled water to the methanolic solution. The solution was cooled for 12–24 h and then filtered through a medium glass filter. The resultant crystals were dried in a vacuum oven at 30–50° overnight.

Spectral analysis. Fluorometric spectra were obtained in a Farrand spectrofluorometer using spectrograde chloroform as solvent. IR spectra, using a Beckman IR-5 spectrometer, were determined according to the "wick stick" procedure outlined by BOBBITT[10] which utilizes pressed KBr sticks. NMR spectra were obtained in formic acid by D. H. GUSTAFSON at Wm. S. Merrell Co., Cincinnati, Ohio, using a Varian A-60 NMR spectrometer. Mass spectrometric analyses were made by the Morgan-Schafer Corp., Montreal, Canada, using a Hitachi Perkin-Elmer RMU-60 mass spectrometer with a direct introduction inlet system.

Results and discussion

Solutions of decoquinate were chromatographed by the analytical TLC method. A freshly prepared methanolic solution gave a single, well-defined zone with a characteristic R_F value of 0.35; analysis of the same solution after 4 h, gave a second, slower moving component ($R_F = 0.17$). The formation of the unknown component was related to the time and the solvent as seen in Table I. Both methanol and chloroform–methanol solutions gave rise to the non-decoquinate material but absolute ethanol did not.

Isolation of the unknown component from methanolic solutions was achieved by preparative TLC. With the system described, several preparations of the unknown ranging in amounts from 20 to 100 mg each were made. When the non-decoquinate component was dissolved in absolute ethanol and chromatographed after several hours, both decoquinate and the non-decoquinate component were present. Thus, decoquinate was formed from the unknown component in the presence of ethanol.

Fluorometric and IR spectroscopy did not reveal any major differences between

TABLE I

FORMATION OF THE UNKNOWN COMPONENT FROM DECOQUINATE IN METHANOL, CHLOROFORM, AND CHLOROFORM–METHANOL (1:1) SOLUTIONS

Solvent	*Component*	*Fluorometric reading at time intervals (h)*[a]								
		0	*1*	*2*	*4*	*5*	*6*	*24*	*72*	*168*
Methanol	Decoquinate	47	47	44	47	41	35	47	17	20
	Unknown component	—	—	6	8	7	4	12	21	30
Chloroform-methanol (1:1)	Decoquinate	50	51	49	50	51	50	45	32	21
	Unknown component	—	—	—	—	—	—	8	22	33
Chloroform-methanol (99:1)	Decoquinate	52	50	52	54	53	53	42	47	51
	Unknown component	—	5	5	10	4	3	4	3	4

[a] Corrected for TLC and solvent blanks.

decoquinate and the non-decoquinate material. NMR spectra did show the presence of a methyl group which appeared as a sharp singlet at 4.15 p.p.m. Spectra of decoquinate did not have this singlet.

Samples of decoquinate and the unknown component were further examined using a mass spectrometer with a direct introduction inlet system. The spectrum of decoquinate exhibited major peaks at *m/e* 373, 371, 232, 231, 203, 202 and a molecular ion at 417. The mass spectrum of the unknown component exhibited a molecular ion at *m/e* 403. Only a trace peak was detectable at *m/e* 417. Comparison of the mass spectra revealed that the unknown component has a fragmentation pattern identical to decoquinate except for the molecular ion peak at *m/e* 403 instead of *m/e* 417. The difference of 14 represents a CH_2 unit.

Structure of the unknown was established from the NMR, mass spectra, and chromatographic properties of the synthesized authentic methyl ester analogue of decoquinate. The properties were identical to those of the unknown.

The results can be described in the following equation where R is the etherified 4-hydroxy quinoline moiety:

$$R\overset{\overset{O}{\|}}{C}OCH_2CH_3 + CH_3OH \longleftrightarrow R\overset{\overset{O}{\|}}{C}OCH_3 + CH_3CH_2OH$$

Other esters of quinoline-3-carboxylic acids undergo this easy transesterification which can be detected by TLC. Solutions of buquinolate (ethyl-4-hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate) in methanol and methyl benzoquinate (7-benzyloxy-6-*n*-butyl-3-methoxycarbonyl-quinol-4-one) in ethanol gave a second component when chromatographed on Silica Gel G using toluene–ethanol–acetic acid as the solvent system.

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