experimental conditions. However, under conditions where the membrane may cover the cornea or by its movement may mechanically remove instilled materials, *e.g.*, suspensions and inserts, absorption kinetics may possibly be altered.

Miller and O'Conner (4) found that dexamethasone sodium phosphate inhibited limbal wound healing in normal rabbit eyes but did not do so when the nictitating membrane had been surgically removed. Our results, as well as the results obtained by Miller and O'Conner, indicate that the influence of the membrane may vary for each preparation and/or experimental condition.

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Determinations of Amygdalinamide, Amygdalin Acid, and 2-Propanol in Amygdalin Dosage Forms from Mexico

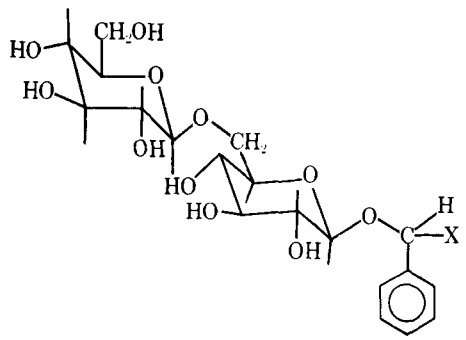
Keyphrases □ Amygdalinamide—synthesized, high-pressure liquid chromatographic analysis in amygdalin dosage forms □ Amygdalin acid—synthesized, high-pressure liquid chromatographic analysis in amygdalin dosage forms □ 2-Propanol—GLC analysis in amygdalin dosage forms □ Impurities—amygdalinamide, amygdalin acid, and 2-propanol, analyses in amygdalin dosage forms □ High-pressure liquid chromatography—analyses, amygdalinamide and amygdalin acid in amygdalin dosage forms

To the Editor:

Assay results for amygdalin dosage forms from Mexico were reported earlier (1). In addition to amygdalin[1] (I), the injectable dosage forms contain significant amounts of amygdalinamide[1] (II) and amygdalin acid[1] (III), hydrolysis products of amygdalin, plus 2-propanol. This communication reports the determination of these three impurities in the amygdalin dosage forms.

Amygdalinamide and amygdalin acid in the amygdalin dosage forms were quantified by an assay that required reference standards of the amide and acid of known purity. Since these standards were unavailable commercially, they were prepared.

Amygdalinamide was prepared from (*R*)-amygdalin by treatment with ammonium hydroxide. The crude product



I: X = CN

II: X = C(=O)NH$_2$

III: X = C(=O)OH

was purified by a preparative high-performance liquid chromatographic (HPLC) procedure that used a C_{18}-bonded silica[2] column and an aqueous methanol mobile phase. When this mobile phase was changed to 0.1 *M* KH_2PO_4, the purified amygdalinamide was separated into two major components of nearly equal intensity plus two very minor impurities and a trace of another. On the basis of peak enhancements, these impurities were identified as (*R*)- and (*S*)-amygdalins and amygdalin acid, respectively. Identity of the principal components in purified amygdalinamide was based on spectral evidence. IR data show an intense band near 5.95 μm assigned to the primary amide, broad absorptions between 9 and 10 μm assigned to carbohydrate, and bands near 13.2 and 14.3 μm assigned to phenyl. The ^{1}H-NMR data were consistent with the proposed structure. Only one peak was found in the region where the benzylic protons were expected; its area was one-fifth that of the phenyl protons. The ^{13}C-NMR data were consistent for a mixture of (*R*)- and (*S*)-amygdalinamides. The amide carbon appeared as two bands centering near 136.6 ppm[3], the phenyl carbons appeared as a group of at least six bands centering near 129.4 ppm, and the two anomeric carbons in the glucose moieties appeared as three bands centering near 101.9 ppm. The benzylic carbon was among the nonanomeric glucosido carbons, which appeared as a complex of many bands. These observations were consistent with those reported (2) for a mixture of (*R*)- and (*S*)-amygdalins. GLC–mass spectral analysis of the trimethylsilyl derivative on a phenyl methyl silicone column (OV-17) showed only one major peak; the mass spectral data for this peak were consistent for the proposed structure as the octatrimethylsilyl derivative.

The purity of the amygdalinamide sample was estimated at 90 ± 1.5% on the basis of chromatographic and elemental data and the results of an NMR assay[4] with pyrocatechol[5] as the internal standard.

[1] Amygdalin is mandelonitrile-β-D-glucosido-6-β-D-glucoside (I). Amygdalinamide is mandeloamide-β-D-glucosido-6-β-D-glucoside (II). Amygdalin acid is mandelic acid-β-D-glucosido-6-β-D-glucoside (III). In the amygdalin dosage forms, all three materials exist as nearly equal mixtures of the respective (*R*)- and (*S*)-epimers.

[2] Porasil C_{18}.

[3] Relative to tetramethylsilane as 0.00 ppm.

[4] This assay measured the total amount of phenyls in the sample. Since the HPLC data showed approximately 1% phenyl-containing impurities, this amount was subtracted from the total phenyls to give a sample purity of 90 ± 1.5%, which is consistent with the elemental data. The remainder of the sample was presumed to be 8.5% water, an amount derived by difference.

[5] Pyrocatechol, Aldrich Chemical Co., analyzed 99+% pure based on chromatographic, titrimetric, and elemental data.

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Table I—Assay Results for Amygdalin Ampules[a]

U.S. Customs Group (Seizure Date)	Fill Volume, ml/Ampul	Amygdalin, mg/Ampul	Percent Label (3 g/Ampul)	Amygdalinamide, mg/Ampul	Amygdalin Acid, mg/Ampul	2-Propanol, mg/Ampul
74-2504-10919	9.05	1443	48.1	127	79	48
(12/28/1973)	9.30	1202	40.2	53	149	15
	9.00	1442	48.1	129	80	48
	9.05	1222	40.7	42	128	15
	8.90	1420	47.3	126	78	47
	9.20	1478	49.3	130	80	48
Average	9.08	1368	45.6	101	99	37
SD	0.11	122	4.1	42	31	17
75-2504-10716	10.20	1634	54.5	526	437	66
(11/09/1974)	10.20	1592	53.1	491	413	63
	9.80	1541	51.4	486	402	61
	10.00	1594	53.1	493	417	63
	10.00	1602	53.4	485	406	64
Average	10.06	1592	53.1	496	415	63
SD	0.18	33	1.1	17	14	2
75-2504-11744	10.05	1267	42.2	704	448	157
(05/21/1975)	9.80	1311	43.7	666	430	149
	9.90	1308	43.6	674	436	148
	11.20	1529	51.0	693	471	165
	10.20	1331	44.4	718	468	160
	10.00	1353	45.1	706	443	153
Average	10.19	1350	45.0	694	450	155
SD	0.33	92	3.1	20	17	7
76-2505-11569	9.65	1527	50.9	669	355	171
(04/11/1975)	10.05	1122	37.4	800	372	189
	9.85	1598	53.3	683	392	173
	10.05	1118	37.3	727	336	180
	10.00	1640	54.7	690	392	174
Average	9.92	1401	46.7	714	369	177
SD	0.17	260	8.7	53	24	7

[a] Relative to the weight of amygdalin in the same ampul, the weight of amygdalinamide ranged from 3 to 71%, the weight of amygdalin acid ranged from 5 to 35%, and the weight of 2-propanol ranged from 1 to 17%. Amygdalinamide and amygdalin acid are hydrolysis products believed to have formed during the autoclave process in the manufacturing scheme. 2-Propanol was probably the extraction solvent used to isolate amygdalin from apricot kernels and similar starting materials.

Amygdalin acid was also prepared from (*R*)-amygdalin by treatment with ammonium hydroxide. The crude product was purified by the preparative HPLC procedure used to purify amygdalinamide, except that the mobile phase was altered slightly to optimize separations. IR data for the purified sample showed bands near 6.29 and 7.13 μm assigned to the carboxylate ion, broad absorptions between 9 and 10 μm consistent with carbohydrate, and bands near 13.2 and 14.3 μm assigned to phenyl.

Its ^{1}H-NMR spectrum showed only one peak in the region where benzylic protons were expected; the ratio of its area to the area of the phenyl protons was 1:5. The ^{13}C-NMR data were consistent with a mixture of (*R*)- and (*S*)-amygdalin acids. This conclusion was based on observations that two signals centering near 138.2 ppm were found for the carboxylate carbon, at least five bands centering near 129.1 ppm were found for the phenyl carbons, and at least three peaks centering near 101.7 ppm were found for the two anomeric carbons in the glucose moieties. The benzylic carbon appeared among the nonanomeric glucosido carbons; this pattern was analogous to the benzylic carbons in (*R*)- and (*S*)-amygdalins and (*R*)- and (*S*)-amygdalinamides. These observations were consistent with those reported (2) for mixtures of (*R*)- and (*S*)-amygdalins.

Derivatized as the pertrimethylsilyl derivative and chromatographed on a phenyl methyl silicone (OV-17) column, the epimeric amygdalin acids coeluted. The mass spectral data for this peak were consistent with the proposed structure as the pertrimethylsilyl derivative. Chromatographed on a C_{18}-bonded silica column coupled to a UV detector and with a mobile phase of 0.1 *M* KH_2PO_4, the epimeric acids were readily resolved into two major peaks of nearly equal area plus three very small, faster eluting impurities that were not precursors to the acid.

The purity of the amygdalin acid sample was estimated at 89 ± 2% (free acid) on the basis of chromatographic and elemental data and the results from an NMR assay, the same procedure used to assay the amygdalinamide.

The source and history of the subject amygdalin dosage forms were described previously (1). However, the individual ampuls used in this study were from a different sampling than those used earlier (1), so some discrepancies will be apparent.

Determinations of amygdalinamide and amygdalin acid were based on HPLC analysis on a C_8-bonded silica[6] column with 0.1 *M* KH_2PO_4–methanol (96:4) as the mobile phase. Aliquots of 1.00 ml from each ampul were transferred to separate 25-ml volumetric flasks, which were then filled to the mark with water; 1.00-ml aliquots of the resulting solutions were each mixed with 1.00 ml of an internal standard solution[7] before being injected[8]. The concentrations of amygdalinamide and amygdalin acid per milliliter were determined by the internal standard method; the amide and acid reference standards were those described earlier in this report. The amounts of amygdalinamide and amygdalin acid per ampul were obtained by

[6] Lichrosorb RP8.

[7] This internal standard solution was prepared by dissolving 3.8 mg of 2-acetylbenzoic acid in 50 ml of 10% methanol in water.

[8] Because of lower concentrations of amygdalinamide and amygdalin acid in ampuls from Group 74-2504-10919, 1.00-ml aliquots from these ampul solutions were each transferred to separate 10-ml volumetric flasks. The remainder of the sample preparation procedure was identical to that for other group numbers except that the internal standard solution was prepared with 1.56 mg instead of 3.8 mg of 2-acetylbenzoic acid.

multiplying the ampul solution volume[9] times the respective solute concentration.

To gain a perspective on the relative amounts of amygdalin and the subject hydrolysis products in a given ampul, the amounts of amygdalin in the respective ampuls also were determined by the previously reported procedure (1).

The determination of 2-propanol was based on a GLC procedure with a porous polymer[10] column coupled to a flame-ionization detector. Aliquots of 0.50 ml from each ampul were each mixed with 0.50 ml of an internal standard solution[11] before being injected. The results derived were compared to a working curve obtained from standard solutions containing known amounts of 2-propanol and 2-butanol in water. The amount of 2-propanol in each ampul was calculated by multiplying its concentration times the ampul solution volume.

The amounts of amygdalin, amygdalinamide, amygdalin acid, and 2-propanol per ampul are listed in Table I.

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[9] Each ampul fill volume was determined by carefully scoring the liquid level in each ampul, emptying and drying the ampul, and refilling to the scored mark with water dispensed from a volumetric buret.

[10] Chromosorb 105.

[11] This internal standard solution was prepared by dissolving 400 mg of 2-butanol in 50 ml of water.

Tolbutamide Binding to Plasma Proteins of Young and Old Human Subjects

Keyphrases □ Tolbutamide—plasma protein binding in humans, effect of subject age □ Binding, plasma protein—tolbutamide in humans, effect of subject age □ Age—of subject, effect on plasma protein binding of tolbutamide in humans □ Antidiabetic agents—tolbutamide, plasma protein binding in humans, effect of subject age

To the Editor:

Some evidence supports the view that age may be an important determinant in drug pharmacokinetics and pharmacodynamics (1). Changes in plasma protein binding of some drugs were shown to account for the age-related differences in their pharmacokinetics. Diminished binding of meperidine, phenylbutazone, warfarin, and phenytoin was associated with decreased serum albumin concentrations in elderly subjects (2–5). Tolbutamide is an acidic drug that is highly bound primarily to the albumin fraction of plasma protein (6, 7).

Table I—Effect of Age on Protein and Albumin Concentrations and Unbound Fraction of Tolbutamide [a]

	Young Group (n = 24)	Old Group (n = 19)
Age, years	38.74 ± 10.76	72.05 ± 8.50
Weight, kg	79.37 ± 2.14	74.25 ± 1.91[b]
Serum protein concentration, g/100 ml	7.25 ± 0.11	7.22 ± 0.51
Serum albumin concentration, g/100 ml	5.25 ± 0.54	4.67 ± 0.65[b]
Unbound fraction of tolbutamide[c]	0.032 ± 0.006	0.040 ± 0.007[d]

[a] Mean ± *SD*. [b] Significantly different ($p < 0.01$; t test). [c] Total plasma tolbutamide concentration of 100 μg/ml. [d] Significantly different ($p < 0.001$; t test).

The present study was undertaken to investigate the effect of age on plasma protein binding of tolbutamide. Blood samples were collected from healthy, nonsmoking, drug-free adult males[1]. The subjects were divided into two groups. The young group (n = 24) ranged in age from 23 to 57 years, and the old group (n = 19) ranged in age from 61 to 87 years.

Protein binding studies were carried out in rigid, clear acrylic cells[2] separated into two 1-ml compartments by a cellophane membrane[3]. Plasma (0.9 ml) was placed into one compartment and then spiked with a 10-μl aliquot of ^{35}S-tolbutamide[4] (dissolved in absolute ethanol) to produce a total concentration of 100 μg/ml. A 0.9-ml volume of 0.067 M phosphate buffer (pH 7.4) was placed in the other compartment.

The cells were placed in a metabolic incubator shaker[5] set at 37° and 50 oscillations/min. At equilibrium (16 hr), 50-μl aliquots were sampled from both compartments and counted directly in a liquid scintillation counter[6]. The unbound fraction of tolbutamide was calculated by dividing the amount of radioactivity (counts per minute) in the buffer solution by that of the plasma sample.

While the albumin concentration was decreased ($p < 0.01$) by about 11% in the old group, there was no change in the total protein concentration (Table I). Similar results were observed previously (9, 10), and the lack of change in the total protein concentration was attributed to a rise in globulin concentration with age. The effect of these changes in protein composition on drug binding has not been predictable (11). Table I indicates that the unbound fraction of tolbutamide was increased ($p < 0.001$) by about 25% in the old group. This age-related difference in the plasma protein binding of the drug prompted a study of its pharmacokinetics in the same groups of subjects. Preliminary results indicate that the total plasma clearance and volume of distribution of tolbutamide increased significantly with age (12). These findings may provide a reasonable explanation for the high incidence of hypo-

[1] Volunteer participants in the Baltimore Longitudinal Study of Aging (8) at the Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, Md.

[2] Fisher Scientific Co., Silver Spring, MD 20910.

[3] Union Carbide Corp., Chicago, IL 60638.

[4] Lot 1076/N19015 (specific activity 4.86 mCi/mmole), Amersham-Searle, Arlington Heights, IL 60005.

[5] Dubnoff, Precision Scientific Co., Chicago, IL 60647.

[6] Packard 2425, Packard Instrument Co., Downers Grove, IL 60515.

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