bon bonded to an asymmetric carbon, and appear as a pair of overlapping quartets.

By combined GLC–mass spectrometry, KI 1595 and 1625 were also shown to represent *N*-methylmonohydroxyethosuximide derivatives (M^+ at m/e 171). The mass spectra of these compounds were virtually identical, indicating a stereoisomeric pair. The most intense peak in both spectra corresponded to loss of C_2H_4O from the molecular ion. In addition, the spectra were devoid of an m/e 143 peak corresponding to loss of C_2H_4 from M^+. On this basis it was concluded that these compounds represented the previously reported (1) stereoisomeric hydroxyethylethosuximides, 2-(1-hydroxyethyl)-2-methylsuccinimide.

Quantitative data were not obtained for the urinary excretion of II. However, its close similarity in structure to the previously reported hydroxyethyl metabolites should result in a nearly 1:1 ratio for isolation and subsequent GLC detection. The results in Fig. 1 thus indicate that II is present in nearly equal concentration as the previously identified hydroxyethyl derivatives. Similar results were obtained for 0–24-hr urine extracts obtained from two male volunteers receiving a single 500-mg dose of ethosuximide. The latter findings indicate that formation of II is not dependent on chronic ingestion of this drug.

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Synthesis and Antibacterial Properties of Substituted Decylbarbituric Acids

JAMES A. BERES[x], TERRI L. COONS, and MILTON J. KAELIN

Abstract □ A series of substituted decyloxybarbituric and decylthiobarbituric acids was prepared and evaluated for antibacterial activity. The acids were synthesized by condensing the appropriate disubstituted malonic ester with urea in a potassium *tert*-butoxide–dimethyl sulfoxide medium at room temperature. When tested against *Bacillus subtilis* and *Staphylococcus aureus*, the most active compound was 5-allyl-5-decylbarbituric acid. A number of other decyloxybarbituric acids showed some activity, but the decylthiobarbituric acids were completely inactive. All compounds were inactive against *Escherichia coli* and *Proteus vulgaris*.

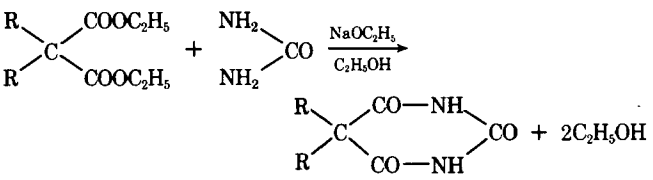
Keyphrases □ Barbituric acids, substituted decyl—synthesized and screened for antibacterial properties □ Decyloxybarbituric and decylthiobarbituric acids—synthesized and screened for antibacterial properties □ Antibacterial activity—substituted decyloxybarbituric and decylthiobarbituric acids

The most general method for the synthesis of barbituric acids has been the base-catalyzed condensation of a malonic ester with urea in refluxing ethanol (1) (Scheme I). Yields from this reaction are normally in the 50–75% range. The advantage of using a potassium *tert*-butoxide–dimethyl sulfoxide medium for this condensation was previously reported (2). This technique was used to synthesize a series of new decyl-substituted barbituric acids which were screened for antibacterial activity. Barbituric acid itself has been shown to inhibit the growth of *Escherichia coli* (3); sodium phenobarbital inhibits the growth of *E. coli*, several hemolytic streptococci, pneumococci, and *Haemophilus influenzae* (4); and several substituted quinolinium barbituric acid salts inhibit the growth of *Streptococcus* and *Staphylococcus* species (5).

RESULTS AND DISCUSSION

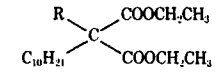
The disubstituted malonic esters (Table I) were prepared by condensing *n*-decyl bromide with the appropriate sodiomalonic ester in dimethylformamide according to a procedure modified from that of Zaugg *et al.* (6). The resulting diesters were then condensed with urea at room temperature in a potassium *tert*-butoxide–dimethyl sulfoxide medium to give the oxybarbituric and thiobarbituric acids (Table II). Of special note are the general improvement of the yields and the simplicity of the reaction workup as compared to the classical technique.

Compounds 6–12 were tested *in vitro* against four microorganisms (Table III): *Bacillus subtilis, Staphylococcus aureus, E. coli,* and *Proteus vulgaris*. The only activity found was against the Gram-positive organisms. All compounds were inactive against the Gram-negative organisms at 250 μg/ml, the highest concentration studied. The most active compound was the 5-allyl-5-decyl derivative, Compound 6. Substitution of sulfur for



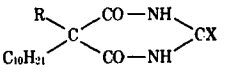
Scheme I

Table I—Substituted Decyl Malonic Esters



Compound	R	Yield, %	Boiling Point (mm)	n_D^{25}	Formula	Analysis, % Calc.	Found
1	Allyl	65	150–152° (0.3)	1.4452	$C_{20}H_{36}O_4$	C 70.55	70.83
						H 10.66	10.73
2	*n*-Butyl	63	154–156° (0.2)	1.4412	$C_{21}H_{40}O_4$	C 70.74	70.45
						H 11.31	11.35
3	Isobutyl	56	154–156° (0.3)	1.4427	$C_{21}H_{40}O_4$	C 70.74	71.15
						H 11.31	11.35
4	Benzyl	46	192–194° (0.3)	1.4695	$C_{24}H_{38}O_4$	C 73.80	73.97
						H 9.81	9.66
5	Methallyl	30	158–160° (0.4)	1.4478	$C_{21}H_{38}O_4$	C 71.12	71.24
						H 10.82	10.64

Table II—Substituted Decylbarbituric Acids



Compound	R	X	Reaction Time, hr	Yield, %[a]	Melting Point[b]	Formula	Analysis, % Calc.	Found
6	Allyl	O	24	89	88–90°	$C_{17}H_{28}N_2O_3$	C 66.21	66.18
							H 9.15	9.08
7	Allyl	S	24	91	87–89°	$C_{17}N_{28}N_2O_2S$	C 62.93	63.12
							H 8.70	8.78
8	*n*-Butyl	O	24	62	93–95°	$C_{18}H_{32}N_2O_3$	C 66.63	66.82
							H 9.94	9.84
9	Isobutyl	O	24	88	61–63°	$C_{18}H_{32}N_2O_3$	C 66.63	66.48
							H 9.94	9.74
10	Benzyl	O	48	78	144–146°	$C_{21}H_{30}N_2O_3$	C 70.36	70.28
							H 8.44	8.44
11	Benzyl	S	48	73	94–96°	$C_{21}H_{30}N_2O_2S$	C 67.34	67.75
							H 8.07	8.18
12	Methallyl	O	48	73	83–84°	$C_{18}H_{30}N_2O_3$	C 67.04	66.79
							H 9.40	9.31

[a] Crude. All compounds were recrystallized from 95% ethanol. [b] Melting points are uncorrected.

oxygen in Compound 6 produced thiobarbituric acid, Compound 7, which was completely inactive. The same substitution with the 5-benzyl-5-decyl derivatives, Compounds 10 and 11, gave a similar effect, although somewhat less dramatic owing to the decreased activity of 10. Although both 5-butyl-5-decyl derivatives were equally active against *B. subtilis*, branching in the butyl side chain, Compound 9 *versus* Compound 8, gave a slightly increased activity against *S. aureus*. Compound 12, combining both the allyl group and the branched chain, was also less active than Compound 6 against *S. aureus*.

EXPERIMENTAL[1]

The synthesis of compounds listed in Tables I and II is illustrated by the following examples.

Diethyl Allyldecylmalonate (Compound 1)—To a suspension of 0.10 mole sodium hydride (4.0 g of a 60% dispersion in mineral oil) in 50 ml dry dimethylformamide was added 20.0 g (0.10 mole) diethyl allylmalonate (slowly over 20 min). After the addition was complete, the mixture was stirred for 1 hr or until hydrogen evolution ceased. Then 23.0 g (0.10 mole) *n*-decyl bromide was added slowly, and the mixture was stirred for 1 additional hr. The reaction mixture was poured into two volumes of ice water and extracted three times with equal volumes of ether. The extracts were dried over magnesium sulfate, the solvent was removed *in vacuo*, and the residue was fractionally distilled *in vacuo* to give 22.0 g (65%) of the malonate, Compound 1, bp 150–152° (0.3 mm). The IR and NMR spectra were fully consistent with structure 1.

[1] Melting points were determined with a Fisher Mel-Temp apparatus and are uncorrected. IR spectra were recorded on an Infracord spectrophotometer, and NMR spectra were measured on a Perkin-Elmer R-20B instrument. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

5-Allyl-5-decylbarbituric Acid (Compound 6)—To a well-stirred suspension of 1.70 g (5 mmoles) Compound 1 and 1.50 g (25 mmoles) urea in 35 ml dry dimethyl sulfoxide was added slowly, with stirring, a solution of 1.22 g (11 mmoles) potassium *tert*-butoxide in 40 ml dry dimethyl sulfoxide. The resulting solution was stirred at room temperature for 24–48 hr, poured into two volumes of an ice-water slush, and acidified with dilute hydrochloric acid. The solid barbiturate (Compound 6) obtained in 89% yield was removed by suction filtration, washed with water, and dried. Recrystallization from 95% ethanol gave white crystals, mp 88–90°; λ_{max} 243 nm, ϵ 10,500 (10^{-3} *M* KOH in ethanol). The IR and NMR spectra were fully consistent with structure 6.

Biological Activity—The antibacterial activity was evaluated using a method modified from that of Waksman and Reilly (7). A stock solution of the compound in methanol was diluted into li-

Table III—Antibacterial Activity of Disubstituted Barbituric Acids

Compound	Minimum Inhibitory Concentration, μg/ml[a] *B. subtilis* ATCC 6633	*S. aureus* ATCC 6538 P	*E. coli* ATCC 10536	*P. vulgaris* ATCC 13315
6	10	10	>250	>250
7	>500	>500	>250	>250
8	10	>500	>250	>250
9	10	350	>250	>250
10	100	250	>250	>250
11	500	>500	>250	>250
12	10	250	>250	>250

[a] Minimum inhibitory concentration is the lowest concentration of compound that prevents visible growth after 48 hr of incubation at 34°.

quefied nutrient agar[2]. After hardening, the plates were inoculated with an aqueous suspension of the test organism, incubated at 34°, and examined after 48 hr.

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COMMUNICATIONS

Antiviral Activity of Triterpenoid Saponins Containing Acylated β-Amyrin Aglycones

Keyphrases □ Triterpenoid saponins containing acylated β-amyrin aglycone structure—antiviral activity □ Antiviral activity—11 triterpenoid saponins containing acylated β-amyrin aglycone, structure evaluated

Sir:

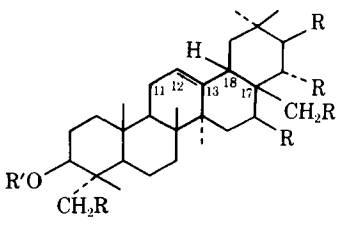
Previously we reported (1) the *in vitro* antiviral activity of gymnemic acids A and B, which were isolated from *Gymnema sylvestre* R. Br. leaves and were shown to be acylated derivatives of β-amyrin glycosides (2–5). Gymnemic acid A also exhibited significant antiviral activity against influenza A virus in mice when administered intraperitoneally (1). The wide occurrence in nature (6) of the triterpenoid saponins containing the β-amyrin skeleton (I) prompted us to evaluate the antiviral activity of this group of natural products. The present communication reports the *in vitro* anti-influenzal activity of 11 naturally occurring triterpenoid saponins structurally related to the gymnemic acids. These results, together with those obtained with gymnemic acid derivatives, permit delineation of preliminary structure–antiviral activity relationships among the triterpenoid saponins studied.

Tables I and II summarize the *in vitro* antiviral activity of gymnemic acids, their derivatives, and various triterpenoid saponins against influenza (A_2/Japan 305) virus. The antiviral activity was evaluated by measuring either the decrease in the 48-hr infectivity yield of virus in eggs from calf kidney cells inoculated with virus (1) or the inhibition of hemadsorption of guinea pig erythrocytes by virus on primary calf kidney cells (15). Based upon the preliminary antiviral results obtained with gymnemic acids (II–V), their derivatives (VI–IX), and other structurally related triterpenoid saponins (X–XX), the following remarks may be made regarding the structure–antiviral activity relationships among the triterpenoid saponins containing β-amyrin skeleton:

1. The majority of the triterpenoid saponins containing the acylated β-amyrin skeleton employed in this study exhibited anti-influenzal activity *in vitro*.

2. The presence of aldehyde (XI, XIV, and XVI) and/or carboxyl (XII, XV, XVI, and XIX) groups in place of or in addition to acyl groups in the β-amyrin skeleton of triterpenoid saponins markedly reduced their antiviral activity.

3. Alteration of the basic β-amyrin skeleton (I), *e.g.*, replacement of the 12,13-double bond with 11,13(18)-diene as in saikosaponin b (XVIII), completely abolished *in vitro* anti-influenzal activity. However, the activity is restored if the 11,12-double bond and methyleneoxy group at the 13,17-position



I: R = R′ = H
VII: R = OH, R′ = H
IX: R = $OCOCH_3$, R′ = $COCH_3$