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Syntheses and Biological Activities of 7-Ethyl-8-chloro-10-(1′-D-ribityl)isoalloxazine and 7-Chloro-8-ethyl-10-(1′-D-ribityl)isoalloxazine, Analogs of Riboflavin†

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The riboflavin analogs 7-ethyl-8-chloro-10-(1′-D-ribityl)isoalloxazine and 7-chloro-8-ethyl-10-(1′-D-ribityl)-isoalloxazine have been synthesized starting with *o*-aminoethylbenzene. The former analog is devoid of biological activity in the riboflavin-deficient rat but it was found to be a strong, reversible antagonist of riboflavin in *Lactobacillus casei*. The latter analog produced good growth response and caused the complete recovery of the visually observable signs of deficiency in the riboflavin-deficient rat and thus shows that it possesses riboflavin-like activity. The latter analog was found to be a potent antagonist in the rat in that when 150 μg/day or more was administered, most of the experimental animals died. This analog also inhibited the action of administered riboflavin in the growth of the rat. Both the lethality of and the inhibition of the growth-promoting properties of riboflavin by this analog could be counteracted by the administration of sufficient riboflavin, thus demonstrating that these activities are properties of an antagonist of riboflavin. The latter analog was found to be a strong, reversible antagonist of riboflavin in *L. casei*.

When a young riboflavin-deficient rat is given riboflavin, it increases its food consumption, improves its food utilization, increases in weight, lives longer, and shows recovery from the visually observable signs of the deficiency. When riboflavin is given with adequate food, these responses are always intimately linked in such animals. If following the administration of any chemical compound, especially if it be an analog of riboflavin, one observes one or more of the above changes to take place, it is entirely reasonable to attribute to such a compound riboflavin-like or vitamin-like activity. If following the administration of such a compound the animal's condition with respect to one or more of the above criteria is worsened, it is reasonable to suspect that the compound might be an antagonist of riboflavin. However, if the activities of the suspected antagonist are prevented or reversed by the simultaneous administration of riboflavin, it is entirely reasonable to ascribe antiriboflavin or riboflavin antagonistic properties to the compound.

This report, as well as others we have published and will publish in the future, has as an important aim the recording of overwhelming evidence that among homologs and analogs of riboflavin which we have synthesized are some possessing almost exclusively riboflavin-like properties. Others possess almost exclusively riboflavin antagonistic activities, and still others which now are several in number clearly display mixed properties, both vitamin-like with regard to some of the above criteria and riboflavin antagonist properties with regard to some of the above criteria.

For an analog of riboflavin to possess significant biological activity it must bear a D-ribityl side chain at position 10 of the isoalloxazine or flavin nucleus. Starting with that basic structural unit, alterations of the substituents at positions 7 and 8 lead to compounds with activities ranging from zero to extremely potent vitamin-like or antagonistic activities. For a clear understanding of this report it will be necessary to review briefly the findings reported for compounds possessing combinations of alkyl and halogen groups in these two positions.

Dichlororiboflavin [7,8-dichloro-10-(1′-D-ribityl)isoalloxazine] was synthesized specifically to test it for riboflavin antagonist properties.[1,2] It proved, however, to be devoid

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‡Killed in action as a combat medic near Da Nang, Vietnam, Sept 19, 1969.

of biological activity for *Lactobacillus casei*[2,3] and for the rat.[2] Dibromoriboflavin [7,8-dibromo-10-(1′-D-ribityl)isoalloxazine] was synthesized[4] for the same purpose and was also found to be inactive. Other halogen-containing analogs of riboflavin which were substituted at positions 7 and 8 have been synthesized for testing, but since the side chain in position 10 was one or another polyhydric alcohol other than D-ribityl, none of these analogs showed activity for *L. casei* or the rat.[5-7]

When the methyl group at either position 7 or position 8 of riboflavin (A) is replaced by an ethyl group as found in 7-ethyl-8-methyl-10-(1′-D-ribityl)isoalloxazine[8] (7-ethyl-8-methylflavin, B) and 7-methyl-8-ethyl-10-(1′-D-ribityl)isoalloxazine[8] (7-methyl-8-ethylflavin, C), most of the vitamin-like activity of the molecule is retained. These two flavins have been found to be indistinguishable from riboflavin in the nutrition of *L. casei* when either is the only flavin available to this microorganism throughout limiting concentration ranges.[9] These two flavins are also able to satisfy the requirement for a flavin in the growth, survival, and good health of the rat. The 7-ethyl-8-methylflavin and the 7-methyl-8-ethylflavin have 47 and 36%, respectively, of the activity of riboflavin for growth of this animal.[9]

Replacement of both of the methyl groups by ethyl groups as found in 7,8-diethyl-10-(1′-D-ribityl)isoalloxazine[10] (7,8-diethylriboflavin, D) results in a flavin possessing full vitamin-like activity for *L. casei*[11] and *Bacillus lactis acidi*[12] but one which is the most potent competitive inhibitor of riboflavin in the rat to have been described to date.[12]

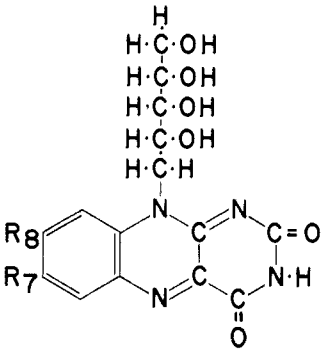
When the 7-methyl group of riboflavin is replaced by a chloro group as in 7-chloro-8-methyl-10-(1′-D-ribityl)isoalloxazine[13] (7-chloro-8-methylflavin, E), the flavin is a potent reversible antagonist of riboflavin in *L. casei;* it is the second most potent antagonist in this system to have been described[14] (I.I. 76).§ This flavin had 50% of the activity of riboflavin as a stimulus for growth of the riboflavin-deficient rat, but at all quantitites given it displays its antagonistic properties in that it kills some of the rats; when larger amounts are given (50–500 μg/day) it kills most to all of the rats. Since this lethal antagonism can be counteracted by riboflavin, the flavin is a reversible antagonist of riboflavin in the rat.[14] The riboflavin-deficient rat shows the following responses when given 7-chloro-8-methylflavin: (a) immediate increase in food consumption; (b) an immediate and sustained increase in weight; (c) recovery of all visually observable manifestations of the deficient state; and (d) efficiency of utilization of food equivalent to that elicited by riboflavin.[15,16] All of these activities are also the normal response to administered riboflavin.

When the 8-methyl group of riboflavin is replaced by a chloro group as in 7-methyl-8-chloro-10-(1′-D-ribityl)isoalloxazine[13] (7-methyl-8-chloroflavin, F), the flavin is the most potent reversible antagonist of riboflavin in *L. casei* to have been described to date[2] (I.I. 59). This flavin stimulates only a small growth response and only a small improvement in appearance of the riboflavin-deficient rat. When administered simultaneously with dietary riboflavin, it interferes with the utilization of the latter and is, therefore, an antagonist, although a weak one.

Replacement of both of the methyl groups by chloro groups as found in 7,8-dichloro-10-(1′-D-ribityl)isoalloxazine[1,2] (dichlororiboflavin, G) results in a flavin which is devoid of biological activity as mentioned in the introduction.

The remarkable biological properties of these two sets of flavins, B and C, where an ethyl group replaced a methyl group, D and E, where a chloro group replaced a methyl group, prompted us to prepare for investigation those two flavins which represented a blend of the two groups. For this reason 7-ethyl-8-chloro-10-(1′-D-ribityl)isoalloxazine (7-ethyl-8-chloroflavin, XV) and 7-chloro-8-ethyl-10-(1′-D-ribityl)isoalloxazine (7-chloro-8-ethylflavin, XVI) have been synthesized for biological evaluation to explore the limits of structural modification consistent with biological activity (Chart I).

Chart I. Basic Isoalloxazine or Flavin Structure



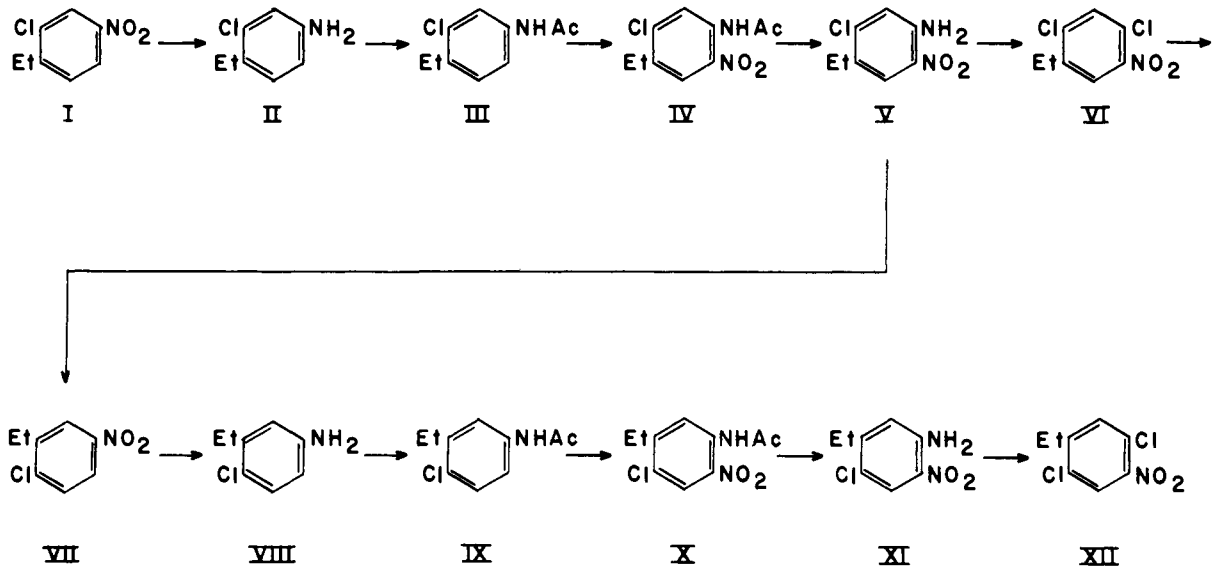
	R_7	R_8	trivial name
A	CH_3-	CH_3-	riboflavin
B	C_2H_5-	CH_3-	7-ethyl-8-methylflavin
C	CH_3-	C_2H_5-	7-methyl-8-ethylflavin
D	C_2H_5-	C_2H_5-	7,8-diethylriboflavin
E	Cl-	CH_3-	7-chloro-8-methylflavin
F	CH_3-	Cl-	7-methyl-8-chloroflavin
G	Cl-	Cl-	dichlororiboflavin
XV	C_2H_5-	Cl-	7-ethyl-8-chloroflavin
XVI	Cl-	C_2H_5-	7-chloro-8-ethylflavin

Chemistry. The synthesis of 7-chloro-8-ethyl- and 7-ethyl-8-chloro-10-(1′-D-ribityl)isoalloxazine required 2-chloro-5-nitroethylbenzene and 2-chloro-4-nitroethylbenzene, respectively. Nitration of *o*-chloroethylbenzene gave a mixture (75% yield), bp 148–152° (21 mm). Analysis of the material by glc revealed three peaks; the fastest to slowest consisting of 17 (narrow band), 58 (broad band), and 25% (narrow band), respectively. Chromic acid oxidation of the mixed nitration product produced the corresponding benzoic acid derivatives, in order of the largest t the smallest amount, of 2-chloro-5-nitro-, 2-chloro-4-nitro-, 2-chloro-3-nitro-, and 2-chloro-6-nitroethylbenzene, respectively. Efforts to separate the mixture of nitro compounds, as well as the mixture of anilines resulting from reduction, and the mixture of acetanilides prepared from the anilines, were unsuccessful. Later, when both 2-chloro-5-nitro- and 2-chloro-4-nitroethylbenzene were available in pure form it was found that they migrated at the same rate in the glc system used and that when mixed they appeared together in the second peak (broad band) referred to above.

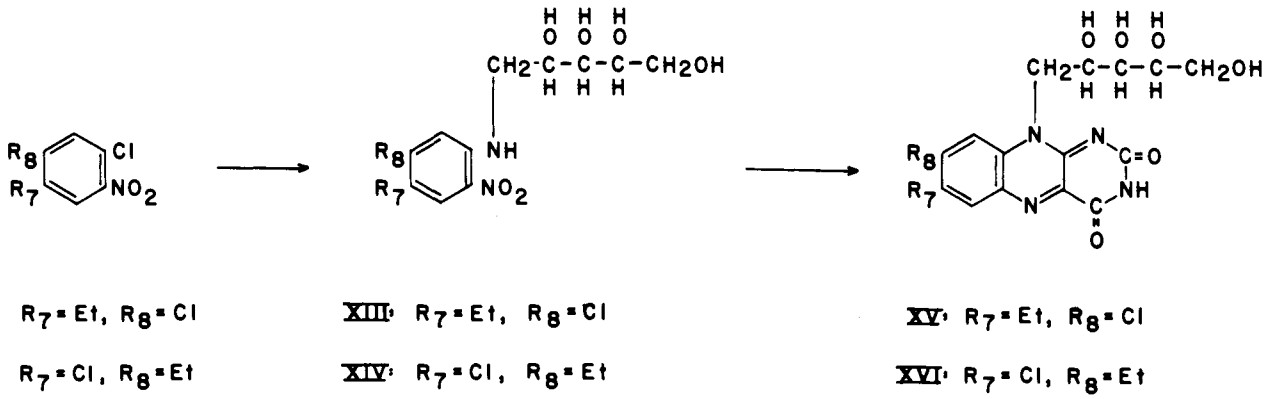
Chlorination of *p*-nitroethylbenzene gave a mixture (85% yield), bp 145–148° (19 mm). Analysis by glc revealed the mixture to consist of 83% of 2-chloro-4-nitro- and 17% of 3-chloro-4-nitroethylbenzene. Fractionation of this mixture produced pure (glc) 2-chloro-4-nitroethylbenzene. A far less tedious procedure for the preparation of 2-chloro-4-nitroethylbenzene was the nitration of *o*-ethylaniline to yield 2-ethyl-5-nitroaniline which was converted to 2-chloro-4-nitroethylbenzene by replacement of the amino group by

§Inhibition index (I.I.) = (μg of analog at half max acid production)/(0.3 μg of riboflavin) × (mol wt of riboflavin)/(mol wt of analog).

Scheme I



Scheme II



VI: R_7 = Et, R_8 = Cl
XII: R_7 = Cl, R_8 = Et
XIII: R_7 = Et, R_8 = Cl
XIV: R_7 = Cl, R_8 = Et
XV: R_7 = Et, R_8 = Cl
XVI: R_7 = Cl, R_8 = Et

chloro group. The 2-chloro-4-nitroethylbenzene also served as the source of 2-chloro-5-nitroethylbenzene as shown in Scheme I and in the Experimental Section.

After the preparation of the required chloronitroethylbenzenes had been completed, it was reported[17] that the nitration of *o*-chloroethylbenzene yielded a mixture of two parts of 2-chloro-5-nitroethylbenzene and one part of 2-chloro-4-nitroethylbenzene, but no separation was undertaken. We found four products to result from this nitration. The same authors reported that the chlorination of *p*-nitroethylbenzene yielded exclusively 2-chloro-4-nitroethylbenzene; we found two products to result from this chlorination.

Once the syntheses of 2-chloro-5-nitroethylbenzene and 2-chloro-4-nitroethylbenzene in pure form had been accomplished, the corresponding 7-chloro-8-ethyl- and 7-ethyl-8-chloro-10-(1′-D-ribityl)isoalloxazines were synthesized as outlined in Schemes I and II. The many steps in the syntheses were essentially like those familiar to us from earlier work[8,13] and only significant differences are noted in the Experimental Section.

Biology. Figure 1 shows the results of the rat growth studies in response to the administration of 7-chloro-8-ethylflavin. When this analog was administered to riboflavin-deficient rats in quantities varying from 50 to 250 μg/day, all the animals grew. Of those receiving 150 μg/day, 40%, and of those receiving 250 μg/day, only 10%, survived the 28-day test period, respectively. The large quantities of the analog required for the growth response demonstrate that it possesses approximately 10% of the activity of the corresponding 7-chloro-8-methylflavin and about 5% of the activity of riboflavin for growth. The inhibitory properties of the analog are shown by the effect of the administration of 250 μg/day plus 20 μg/day of riboflavin, where the growth response was found to be the same as that elicited by 10 μg/day of riboflavin. Half of the animals receiving 250 μg of analog and 20 μg of riboflavin/day survived the test period. The lethal properties of 250 μg/day of the analog are counteracted by the simultaneous administration of 40 μg/day of riboflavin; the growth response is reduced, however, to the response elicited by the administration of 20 μg/day of riboflavin. During the administration of 7-chloro-8-ethylflavin, the riboflavin-deficient rats showed complete recovery from the visually observable signs of the deficiency.

The response of *L. casei* to 7-chloro-8-ethylflavin is shown in Figure 2. As a replacement for riboflavin in the metabolism of this microorganism, the analog is inert. As an inhibitor of the utilization of riboflavin by this organism, it is a strong, reversible inhibitor, I.I. 101.

More acid was produced in tubes containing small quantities of analog and riboflavin than could have been produced by the utilization of riboflavin alone. Presumably, this additional acid was produced by the mutant strain of *L. casei* which we have discovered and isolated.[14,18,19]

Table I shows the results of the administration of 7-ethyl-8-chloroflavin to riboflavin-deficient rats. When the animals were given various quantities up to 1 mg/day, the rats

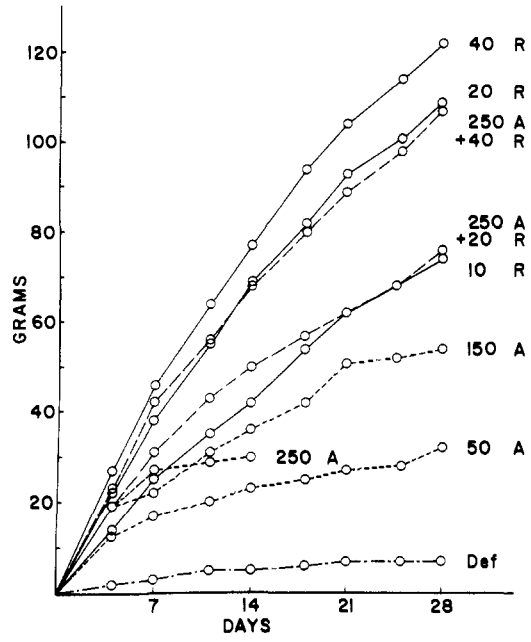


Figure 1. Growth of rats administered 7-chloro-8-ethylflavin, riboflavin, or mixtures of the two flavins: O———O, riboflavin; O— —O, analog plus riboflavin; O- - - -O, analog; O— - —O, deficient controls; A, analog (7-chloro-8-ethylflavin); R, riboflavin. The numbers associated with A and R are in μg. For the 250-μg A, 150-μg A, and 250-μg A plus 20-μg R, 10, 40, and 60%, respectively, of the animals survived the 28-day test period. All other groups had 100% survival. Standard errors of the means are of the same order of magnitude as those in Table I.

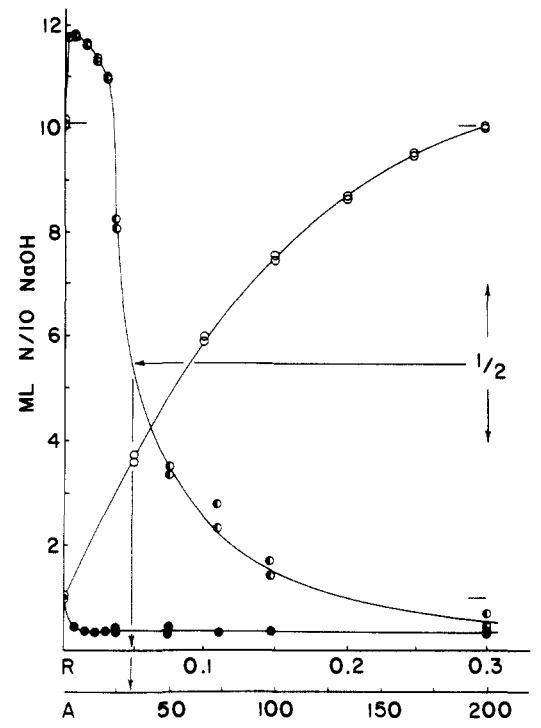


Figure 2. Lactic acid production by *L. casei* grown in a culture medium containing riboflavin (empty circles), 7-chloro-8-ethylflavin (filled circles), and mixtures of riboflavin and the analog (half-filled circles): R, riboflavin; A, analog; the quantities of flavins are in μg/tube. For the mixed flavin curve (half-filled circles) all tubes contain 0.3 μg of R and from 0 to 200 μg of A. The response to 0.3 μg of R was reduced to 50% when 33 μg of A was also present in the tube. I.I. = 33/0.3 × 376/411 = 101.

showed no increase in food consumption, no growth response, no improvement in their appearance, and no worsening of their deficiency state. The analog is, therefore, inert for the rat either as a vitamin replacement or as an antagonist of riboflavin.

The response of *L. casei* to 7-ethyl-8-chloroflavin is shown in Figure 3. As a replacement for riboflavin in the metabolism of this microorganism, this analog is also inert. When made available to the microorganism in the presence of riboflavin throughout the range where the ratio of analog to riboflavin was 10:1 to 70:1, the analog is exceedingly stimulatory in the sense that a large excess of lactic acid is produced. Presumably, this additional acid was also produced by the mutant strain of *L. casei* referred to above. At ratios of analog to riboflavin of from 80:1 to 150:1 the material is a strong reversible inhibitor; as the data show it possesses an I.I. of 105.

7-Ethyl-8-chloroflavin, as was found to be true for 7-methyl-8-chloroflavin[2] and 7-methyl-8-bromoflavin,[20] possesses an E'_0 which is sufficiently positive so that it is reduced to a "poised" rhodoflavin state when autoclaved with the basal medium used for the growth of *L. casei*.

Table I. Growth of Rats Administered 7-Ethyl-8-chloroflavin for 28 Days

Group	Daily supplement, μg	Worsened appearance,[a] %	Wt gained,[b] g ± S.E.		
1	0	100	6 ± 2		
				(0.731)[c]	
2	20	100	7 ±2		
				(0.142)	
3	50	60	10 ± 1		(0.145)[d]
				(0.174)	
4	250	90	8 ± 2		
				(0.717)	
5	1000	90	7 ± 2		

[a] The percentage of the animals in a group whose appearance was worse at day 28 than at day 0. [b] The average weight for all groups when the animals had become deficient and started on supplementation was 66 ± 3 g. [c] *P* value for difference of means of two groups in lines immediately above and below the values in parentheses. [d] *P* value for differences of means (largest differences) for groups 1 and 3.

Discussion

The replacement of the methyl group in either position 7 or 8 of riboflavin by an ethyl group produces analogs which are vitamin-like for both rats and *L. casei*. The replacement of either of the position 7 or 8 methyl groups by a chloro group produces analogs which are both vitamin-like in certain of the responses elicited and antagonistic in certain other responses elicited in the rat, and both are antagonistic for the wild strain of *L. casei*. When one of the methyl groups in positions 7 or 8 of the vitamin is replaced by an ethyl group and the other by a chloro group, we have begun to reach the limits of modifications in the riboflavin structure associated with some form of biological activity. 7-Chloro-8-ethylflavin possesses some of both the vitamin-like and inhibitory properties for the rat, although both are shown to be of greatly reduced potency when compared with 7-chloro-8-methylflavin. It possesses strong potency as an antagonist of riboflavin in *L. casei*. The isomeric 7-ethyl-8-chloroflavin is devoid of biological activity for the rat although it possesses strong potency as an antagonist of riboflavin in *L. casei*.

Experimental Section

Chemistry. Melting points were determined in open Pyrex capillary tubes in an electrically heated, modified Drechsel-type

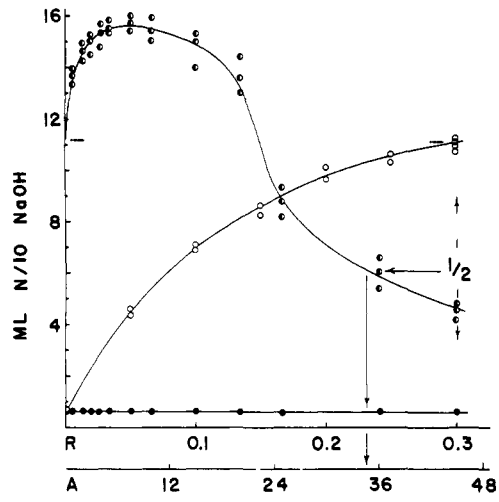


Figure 3. Lactic acid production by *L. casei* grown in a culture medium containing riboflavin (empty circles), 7-ethyl-8-chloroflavin (filled circles), and mixtures of riboflavin and the analog (half-filled circles): R, riboflavin; A, analog; the quantities of flavins are in μg/tube. For the mixed flavin curve (half-filled circles) all tubes contain 0.3 μg of R and from 0 to 45 μg of A. The response to 0.3 μg of R was reduced to 50% when 34.5 μg of A was also present in the tube. I.I. = 34.5/0.3 × 376/411 = 105.

bath and are corrected (thermometers calibrated against U.S.P. melting point reference standards). Boiling points are uncorrected. Decomposition points of the two flavins were obtained by immersing the capillary into the preheated bath (225°) and then heating rapidly to the decomposition point (uncorrected). Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Uv spectra were determined on a Beckman DU equipped with a Gilford photometric indicator unit. Glc were determined on a F & M Model 810; the column was packed with 10% diethylene glycol succinate on 80–100 mesh Chromosorb. R_f values were determined in 1-butanol–water–acetic acid (4:5:1) on Whatman No. 1 by descending technique. The rats were CFN (Carworth, Inc., New City, N. Y.). *L. casei* 7469 was obtained from American Type Culture Collection, Rockville, Md.

Practical grade *o*-ethylaniline (Eastman, p-3066) was nitrated in the early portions of this work as described[21] but later by an improved procedure[17] to produce 2-ethyl-5-nitroaniline (84%) as yellow crystals, mp 63–64° (EtOH) (lit.[17] 63–64°).

2-Chloro-4-nitroethylbenzene (I). (a) 2-Ethyl-5-nitroaniline, 83 g (0.5 mol), was dissolved in a warm solution of 270 ml of concentrated HCl and 400 ml of H_2O and cooled to 0°. A cold solution of 35.7 g (0.515 mol) of $NaNO_2$ in 60 ml of H_2O was added slowly below the surface of the stirred aniline hydrochloride mixture, temperature not above 5°. To a stirred solution of 99 g of CuCl in 300 ml of concentrated HCl, the diazonium solution was added as a small stream. The mixture was steam distilled; the product was isolated in the usual way and distilled under reduced pressure to produce 83–88 g (89–94%) of yellow oil: bp 146–148° (14 mm); 152° (24 mm); glc pure. *Anal.* ($C_8H_8ClNO_2$) C, H, Cl, N.

(b) *p*-Nitroethylbenzene (Eastman, p-2996) was purified by fractionation, only that portion of bp 118–119.5° (10 mm) (glc pure) was utilized. It was chlorinated by the procedure described[22] to produce in 83% yield a mixture shown by glc and chromic acid oxidation to consist of 2-chloro-4-nitroethylbenzene (83%) and 3-chloro-4-nitroethylbenzene (17%), bp 145–148° (19 mm). The mixture was fractionated in a 22-in. Penn State column collecting only that portion of bp 148–149° (18 mm) in 48% overall yield of 2-chloro-4-nitroethylbenzene (glc pure). Products from procedures a and b were identical by glc.

3-Chloro-4-ethylacetanilide (III). I (0.25 mol) was reduced with Fe filings and HCl to yield 3-chloro-4-ethylaniline (II). The reaction mixture was steam distilled to remove unreduced I, made alkaline with NH_3, and then steam distilled to remove the aniline (*II*). Following isolation by usual procedures *II* was obtained as a colorless oil (91–97%): bp 120–121° (9 mm); 130–131° (17 mm). II was converted immediately to the acetanilide with Ac_2O in yields of 74–82% as white plates, mp 101–103° (benzene). *Anal.* ($C_{10}H_{12}ClNO$) C, H, Cl, N.

2-Nitro-4-ethyl-5-chloroacetanilide (IV). III (19.8 g, 0.1 mol) was nitrated by the procedure described for nitration of 4,5-diethyl-2-nitroacetanilide[10] to produce 21.9–22.5 g of crude product which was dissolved in 60 ml of hot EtOH and refrigerated. The product (IV) was obtained: 15.9–16.2 g (65–67%); mp 103–104°. Laborious work-up of the filtrate yielded an additional 2.5 g of mp 102–104° and two by-products, 2.0 g of mp 114–116° and 1.0 g of mp 72–75° A second recrystallization of IV gave yellow needles, mp 104–106° (EtOH). IV sublimes at 56° *in vacuo*. *Anal.* ($C_{10}H_{11}ClN_2O_3$) C, H, Cl, N.

2-Nitro-4-ethyl-5-chloroaniline (V). IV (50 g, 0.205 mol) was hydrolyzed as described for 2-nitro-4-methyl-5-chloroaniline[13] to produce 37.8–40.3 g (92–98%) of V as orange plates, mp 107–108° (1:1 benzene–*n*-hexane). *Anal.* ($C_8H_9ClN_2O_2$) C, H, Cl, N.

2,4-Dichloro-5-ethylnitrobenzene (VI). V (53.7 g, 0.267 mol) was converted to 2,4-dichloro-5-ethylnitrobenzene (VI) as described for the preparation of 4,6-dichloro-3-nitrotoluene[13] to produce 32.3–34.6 g (55–59%) of VI as yellow prisms, mp 45–46° (*n*-hexane) (glc pure). *Anal.* $C_8H_7Cl_2NO_2$) C, H, Cl, N.

2-Chloro-5-nitroethylbenzene (VII). 2-Nitro-4-ethyl-5-chloroaniline (V) (40.2 g, 0.2 mol) was deaminated by the procedure described for deamination of 2-nitro-4-ethyl-5-methylaniline[8] to produce 30.6–31.1 g (83–84%) of VII as a yellow oil: bp 120° (7 mm); 125–126° (9 mm); 136° (15 mm) [lit.[23] 120° (7 mm)]; glc pure. *Anal.* ($C_8H_8ClNO_2$) C, H, Cl, N.

3-Ethyl-4-chloroacetanilide (IX). VII (92.8 g, 0.5 mol) was reduced by the procedure used to reduce I to produce 3-ethyl-4-chloroaniline (VIII), 67.7–69.9 g (87–90%), as a slightly yellow oil: bp 130–131° (12 mm); 141–142° (21 mm). VIII was converted immediately to the acetanilide IX (75–80%) as white plates, mp 96–98° (benzene). *Anal.* ($C_{10}H_{12}ClNO$) C, H, Cl, N.

2-Nitro-4-chloro-5-ethylacetanilide (X). IX (63.0 g, 0.317 mol) was nitrated as described for III to produce X. The crude nitration product, 72.5 g, was dissolved in 130 ml of EtOH to yield on cooling 31.3 g (40%) of product as yellow prisms, mp 100–102°. Laborious work-up of the filtrate yielded an additional 4.3 g (total 46%) and two by-products, 16.2 g, mp 85–88°, and 9.2 g, mp 141–143°. X sublimes at 76° *in vacuo*. *Anal.* ($C_{10}H_{11}ClN_2O_3$) C, H, Cl, N.

2-Nitro-4-chloro-5-ethylaniline (XI). X (35.4 g, 0.145 mol) was hydrolyzed as described for IV to produce XI, 18.7 g (64%), as orange-red prisms, mp 113–115° (1:1 benzene–*n*-hexane). *Anal.* ($C_8H_9ClN_2O_2$) C, H, Cl, N.

2,5-Dichloro-4-ethylnitrobenzene (XII). XI (17.4 g, 0.087 mol) was converted to XII as described for the preparation of VI to produce 15.8 g (83%) of yellow oil: bp 150–151° (8 mm); 154–155° (12 mm); glc pure. *Anal.* ($C_8H_7Cl_2NO_2$) C, H, Cl, N.

2-Nitro-4-ethyl-5-chloro-*N*-D-ribitylaniline (XIII). 2,4-Dichloro-5-ethylnitrobenzene (VI) (5.00 g, 0.023 mol) and 10 g of D-ribamine in 150 ml of pyridine were refluxed for 10 hr and processed as described for 2-nitro-4-methyl-5-chloro-*N*-D-ribitylaniline[13] to produce 4.0–4.3 g (53–57%) of XIII as orange-yellow needles, mp 186–188° (MeOH). *Anal.* ($C_{13}H_{19}ClN_2O_6$) C, H, Cl, N.

2-Nitro-4-chloro-5-ethyl-*N*-D-ribitylaniline (XIV). 2,5-Dichloro-4-ethylnitrobenzene (XII) was allowed to react with D-ribamine as described for VI, except it was refluxed for 24 hr. The product was processed as described to yield 5.2–6.1 g (68–81%) of red-orange prisms, mp 162–163° (EtOH). *Anal.* ($C_{13}H_{19}ClN_2O_6$) C, H, Cl, N.

7-Ethyl-8-chloro-10-(1′-D-ribityl)isoalloxazine (XV). 2-Nitro-4-ethyl-5-chloro-*N*-D-ribitylaniline (XIII) (5.875 g, 0.0143 mol) was converted to XV by essentially the procedure used for the synthesis of 6-methyl-7-chloro-10-(1′-D-ribityl)isoalloxazine[13] except for the following changes. The hydrogenation required 1–2 hr. Following 2 days at room temperature, the reaction mixture which had deposited considerable crystalline material was evaporated to dryness *in vacuo*. Absolute EtOH, 100 ml, was added and evaporated; this treatment repeated three times. The product was suspended in 50 ml of H_2O and collected on a filter and dried to yield 6.22–6.52 g of crude flavin. The product was recrystallized from approximately 800–900 ml of 2% AcOH, and the product was recrystallized again from the minimum required amount of 2% AcOH to yield 4.95–5.68 g (69–79%) of orange-yellow needles: mp 260–264° dec (uncor); R_f 0.55–0.57; λ_{max} (H_2O) 267 (ε 32,255), 365 (9660), 444 (12,255); λ min 302 (1617), 395 (6170). For riboflavin: λ max 267 (32,278), 374 (10,425), 445 (12,317); λ_{min} 306 (1120), 402 (6795). *Anal.* ($C_{17}H_{19}ClN_4O_6$) C, H, Cl, N.

7-Chloro-8-ethyl-10-(1′-D-ribityl) isoalloxazine (XVI). 2-Nitro-4-chloro-5-ethyl-*N*-D-ribitylaniline (XIV) (5.35 g, 0.016 mol) was converted to XVI by the same procedure used to prepare XV to yield 6.14–6.24 g of crude flavin. When recrystallized as described 4.82–4.90 g (74–75%) of yellow needles was produced: mp 260–

262° dec (uncor); R_f 0.56–0.57; λ_{max} (H_2O) 268 (ϵ 38,424), 359 (9852), 446 (12,315); λ_{min} 307 (2266), 391 (5468). *Anal.* ($C_{17}H_{19}ClN_4O_6$) C, H, Cl, N.

Biology. The procedures used for the biological evaluation of the new analogs in rats and *L. casei* were the same as those used by us on earlier occasions[2,14] for similar analogs.

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Cycloalkanones. 2. Synthesis and Biological Activity of α,α'-Dibenzylcycloalkanones

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A number of mono- and disubstituted cycloalkanones have been synthesized by condensation of the cyclic ketones with the appropriate aldehyde in the presence of sodium ethoxide. The hypocholesterolemic activity has been studied and the most potent compound was found to be *trans*-2,8-dibenzylcyclooctanone which showed an ED_{50} of 10 mg/kg per day which lowered rat blood cholesterol levels to 50% of the normal levels.

Research involving studies on the chemotherapy of atherosclerosis has dealt with compounds that lower specific serum lipids, *i.e.*, cholesterol and triglycerides. The present investigation describes the synthesis and initial biological studies on a series of substituted cycloalkanone derivatives. These were tested for hypocholesterolemic effects in rats and mice. The series studied included pentanone, hexanone, heptanone, octanone, nonanone, and dodecanone derivatives. Compound series including cyclohexane and indan derivatives have been studied by others.[1]

Of the cyclic ketones tested, the 2,8-dibenzylcyclooctanone was found to be most effective as a potential hypocholesteremic agent. At the screening dose of 10 mg/kg orally, 2,8-dibenzylcyclooctanone effectively lowered the serum cholesterol levels to 50% as compared to the controls.

Isomeric dimethoxy- and trimethoxybenzylidenecycloalkanones have been reported by Mattu and Manca,[2] and some diarylidenecycloalkanones have been described by Farrell and Read.[3] The photochemical properties of 2,5-dibenzylidene-3-cyclopentanone have been studied by Chapman and Pasto.[4] However, the aryl methylene compounds have not been reported. Recently, Irvine, *et al.*,[5] have investigated the stereochemistry of α,α'-dibenzylcycloalkanones.

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Experimental Section

The disubstituted cycloalkanones were prepared by condensation of cyclic ketones with the appropriate aldehyde in the presence of NaOEt. The resulting dibenzylidene cycloalkanones were then treated with palladium–charcoal catalyst in the presence of EtOAc to give the disubstituted dibenzylcycloalkanone. All melting points are corrected and were obtained on a Mel-Temp apparatus. Elemental analyses were performed by M-H-W Laboratories, Garden City, Mich., or Atlantic Microlab, Atlanta, Ga., and where indicated by symbols were within ±0.4% of the theoretical values.

Clofibrate. The oil from commercial capsules of clofibrate (Atromid-S, Ayerst) was distilled, and the fraction boiling at 145–146° (0.005 mm) was collected.

Cyclopentanone, cyclohexanone, cycloheptanone, cyclododecanone, and cyclononanone were used as received from the supplier after thin-layer chromatography which showed no contaminants.

Cyclooctanone was sublimed at 0.6 mm prior to testing.

2,6-Dibenzylidenecyclohexanone. This compound was prepared according to the published method for dibenzalacetone on a 0.5 *M* scale:[6] yield 91 g (67%); mp 118–121° (lit.[3] 118–119°). *Anal.* ($C_{20}H_{18}O$) C, H.

2,7-Dibenzylidenecycloheptanone. Condensation of cycloheptanone and benzaldehyde was carried out according to the method given below for cyclooctanone: yield 12.4 g (43%); yellow needles from MeOH; mp 106–110° (lit.[3] 107°). *Anal.* ($C_{21}H_{20}O$) C, H.

2,8-Dibenzylidenecyclooctanone. Na (5 g) was dissolved in 125 ml of absolute EtOH. When the solution had cooled to room temperature, 12.6 g (0.1 mol) of cyclooctanone (Aldrich) dissolved in 21.2 g (0.2 mol) of benzaldehyde was added in one batch. There was an immediate rise in temperaure of 10–15°, and formation of a light yellow color in the solution, and a solid soon began to form. After about 3 hr of stirring, the odor of benzaldehyde was still present in the reaction flask but was absent after 4 hr. At this time the reaction mixture was filtered. Filtration was slow (about 45 min) due to the gummy nature of the solid material. The solid was then stirred with approximately 300 ml of water for 30–45 min followed with