in the early stages and levels off at approximately one-half the concentration for the treated samples, in 90 to 100 hours.

Diphenylamine and the Ionone Effect.—Several experiments have been run to determine whether β-ionone could overcome the adverse effect of DPA on carotene production. Results vary with culture age and degree of maturity. A 36-hour culture (6 plates each) was treated with ionone, ionone + DPA, DPA and a fourth set served as control. Twelve hours later, yields of carotene were 38.7, 5.9, 1.1 and 4.1 μg., respectively, per culture set. A 74-hour culture, similarly treated, showed no such effect, averaging 36 μg. carotene per set, though a slight increase, *ca.* 25% was noted on a weight basis. However since both DPA and ionone influence normal culture development, a direct effect of the ionone can only be postulated after DPA treatment if there is an increase in the absolute amount of pigment synthesized *per culture*.

A series of runs were then made in which cultures were incubated for 40 hours, after which different treatments were applied. To eliminate possible differences in the rates of absorption of ionone and DPA, one was applied at stated time intervals after the other. At this stage, much trouble was encountered with the temperature controls. Only the results of Table II are strictly comparable. Those of Table III are comparable horizontally. The physiological ages of the cultures for the latter, at the time of ionone or DPA treatment are not identical, and this is reflected in differences in for example the response to the ionone alone. The DPA treatment, followed by ionone 3 hours later varies also. It seems clear that on a weight basis, the β-ionone overcomes in part the DPA effect. It never equals the ionone-treated samples alone, and it may or more often may not be as effective as the controls. It appears in general to be most effective with young cultures. This of course has been characteristic of the ionone treatment unaffected by the complication of DPA action.

TABLE II

EFFECT OF DPA AND IONONE

Treatment	β-Carotene, μg./5 cultures	μg./g.
β-Ionone	78.5	296
Control	49.5	197
β-Ionone (DPA 3 hr. later)	43.2	238
β-Ionone (DPA 2 hr. later)	35.4	173
β-Ionone (DPA 1 hr. later)	25.5	175
β-Ionone (DPA 0 hr. later)	17.7	128
DPA (β-ionone 1 hr. later)	17.3	111
DPA (β-ionone 2 hr. later)	16.6	126
DPA (β-ionone 3 hr. later)	16.2	113
DPA	14.4	77.4

TABLE III

PIGMENT PRODUCTION AND DPA–IONONE EFFECTS WITH TIME

Time in hr.[c]	Control		Ionone		DPA Alone		DPA + Ionone at once		DPA + Ionone 3 hr. later	
	1[a]	2[b]	1	2	1	2	1	2	1	2
0	2.54	141	...	..	..	...	...	...	..	...
12	36.5	185	100.5	543	17.6	115	...	...	67	438
48	189	401	229	627	29.1	98	51	175	64	192
96	284	710	446	1230	19	58	105	292	95	271

[a] Micrograms carotene per 5 cultures. [b] Micrograms per gram dry mycelium. [c] After addition of ionone, or of DPA alone.

Miscellaneous

Temperature.—It had been thought from visual observation that a greater difference in carotenoid content would be found, between treated and control cultures, if held at 15° than when maintained at 25°. This however proved illusory. Regular 36-hour cultures were subdivided and held at 15° and 25° for an additional 24 hours, with and without ionone. Treated cultures contained 1565 and 2240 μg. of pigment per g., for 15° and 25°, respectively, compared with 119 and 144 for the controls. A second run confirmed a ratio of *ca.* 1.3 in the quantities of carotene produced at the two temperatures, regardless of treatment.

Vitamin A.—A slight yellowing of the culture was noted when vitamin A alcohol or acetate was added to an agar or gelatin medium. The yellowing, shown by chromatography to be due to β-carotene is however only observed when the vitamin A has been added to the medium prior to autoclaving. We steam distilled 0.5 g. of Crystalets (Chas. Pfizer and Co., Crystalline vitamin A acetate stabilized with gelatin and sugar) and obtained 250 ml. of distillate. This was ether-extracted, the extract evaporated and transferred to alcohol. Placed in the usual liquid medium, it enhanced carotene production, as shown by a marked yellowing of the culture during the next 12–24 hours.

Discussion

We feel that any extensive discussion of the above phenomenon must await the outcome of labeled ionone studies. Citral is completely ineffective in promoting carotene synthesis. Whether β-cyclocitral might show a response is not known.

The most attractive hypothesis, essentially a speculation at this stage, is that either β-ionone or a large fragment of it including the ring structure, is incorporated into the carotene molecule.[6] If the probability of this is conceded, it would follow that carotene production in the mold is limited by the rate at which it can effect ring closure of the appropriate intermediate. This is supplied by β-ionone and apparently by breakdown products of vitamin A.

(6) Evidence reported at the Pacific Slope Biochemical Conference, Berkeley, October 11, 1952, indicates that with carbonyl-labeled ionone, the carotene is inactive. Also, ionone has no effect on production of carotene in a nitrogen atmosphere. Two possibilities therefore exist—that a β-ionone fragment is incorporated or that the effect is indirect. It may be added that no effect was obtained with methylheptenone.

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β-Arylserine Ethyl Esters[1]

BY WALTER H. HARTUNG, T. T. DITTRICH AND YEN-TSAI CHANG[2]

RECEIVED FEBRUARY 6, 1952

β-Arylserines are of interest because the 3,4-dihydroxy compound is the hypothetical precursor of the hormones elaborated by the suprarenal medula, *viz.*, norepinephrine and epinephrine; *e.g.*

$$3,4\text{-}(HO)_2C_6H_3\text{—}CHOH\text{—}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{—}COOH \longrightarrow 3,4\text{-}(HO)_2C_6H_3\text{—}CHOH\text{—}CH_2NH_2 + CO_2$$

Perhaps the non-catechol analogs may serve as models with which to examine the prospect of *in vivo* enzymatic decarboxylation to give rise to arylethanolamines, all of which would be expected to exhibit pressor properties.[3]

The following convenient reactions have been used to prepare the ethyl esters of phenyl- and *p*-chlorophenylserine.

(1) Number 13 on Amino Acids. For No. 9 see W. E. Weaver and W. H. Hartung, *J. Org. Chem.*, **15**, 741 (1950).

(2) Fellow American Foundation for Pharmaceutical Education, 1948–1951.

(3) K. H. Beyer, *Advances in Chemistry*, Ser. No. 2, 37 (1950).

$$\text{Ar-COCH}_2\text{COOEt} \longrightarrow \text{ArCOCCOOEt} \longrightarrow \underset{\underset{\text{NOH}}{\|}}{\text{ArCOCCOOEt}} \longrightarrow \text{Ar-CHOH—CH(NH}_2\text{)—ECOOt}$$

The products, capable of existing in two racemic forms, are obtained in the (±)-*erythro*-configuration.[4]

Experimental

Ethyl benzoyloximinoacetate, prepared by treating an ethereal solution of ethyl benzoylacetate with isopropyl nitrite according to the general procedure of Hartung and Munch[5] was obtained in 85–88.5% yields, formed white crystals m.p. 123–124° (uncor.) after two crystallizations from toluene.[6]

Anal. Calcd. for $C_{11}H_{11}O_4$: N, 6.33. Found: N, 6.35, 6.29.

Ethyl ester of *dl-erythro*-β-phenylserine, as obtained by catalytic hydrogenation is described by Chang and Hartung.[7]

Ethyl *p*-chlorobenzoyloximinoacetate was obtained from ethyl *p*-chlorobenzoylacetate and isopropyl nitrite[5] in yields 57–62.5%. Crystallized from toluene, the product melted 135–136° (uncor.).

Anal. Calcd. for $C_{11}H_{10}O_4NCl$: N, 5.49. Found: N, 5.45.

Ethyl ester of *dl-erythro*-β-*p*-chlorophenylserine was obtained by hydrogenating with Pd-on-C catalyst 7.0 g. of the oximino intermediate in 175 ml. ethanol in which was dissolved 20 g. of HCl. The H_2 uptake was 12% more than calculated, and it is possible that some of the chlorine was removed from the phenyl nucleus. Obtained 6.3 g. of the hydrochloride, 82.5%, m.p. 168–170°.

Anal. Calcd. for $C_{11}H_{14}O_3NCl\cdot HCl$: N, 5.22. Found: N, 5.19, 5.30.

(4) The configuration is assigned on the basis of correlation studies by (a) K. N. F. Shaw and S. W. Fox, abstracts, p. 28N, Chicago Meeting, American Chemical Society, 1950; (b) G. Carrara and G. Weitnauer, *Gazz. chim. ital.*, **79**, 856 (1949); (c) D. Billet, *Compt. rend.*, **230**, 1074 (1950); (d) K. Vogler, *Helv. Chim. Acta*, **33**, 2111 (1950).

(5) W. H. Hartung and J. C. Munch, THIS JOURNAL, **51**, 2262 (1929).

(6) A. Bernton, *Arkiv. Kemi Mineral, Geol.*, **7**, No. 13, 1 (1919); *C. A.*, **14**, 2168 (1920), gives m.p. 121°.

(7) Y. T. Chang and W. H. Hartung, THIS JOURNAL, **75**, 89 (1953).

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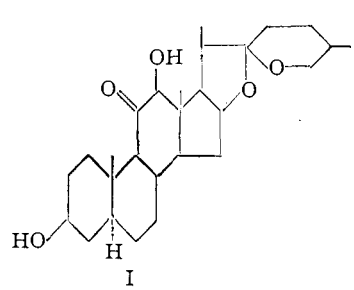
Steroidal Sapogenins. XXV.[1] Experiments in the Hecogenin Series (Part 4).[2] Degradation of 22a-5α-Spirostane-3β,12β-diol-11-one[3]

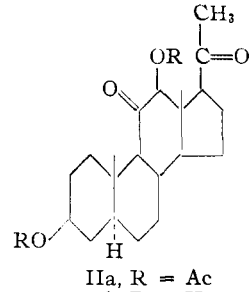
BY H. MARTINEZ, HOWARD J. RINGOLD, G. ROSENKRANZ AND CARL DJERASSI[4]

RECEIVED SEPTEMBER 17, 1952

It has been reported previously[2] that Gallagher's[5] procedure for the shift of the C-12 carbonyl function to position 11 developed in the bile acid series is inapplicable to the sapogenins, since the penultimate step—displacement of the C-12 hydroxyl group of 22a-5α-spirostane-3β,12β-diol-11-one (I)[6] by phosphorus tribromide—fails due to side reactions with the spiroketal system. It was necessary, therefore, to develop an alternate path through bismuth oxide oxidation[2] to an 11,12-dione and subsequent removal of the C-12 carbonyl group. Simultaneously with this work,[2] we have also investigated the feasibility of Gallagher's method[5] in the pregnane series and the present note deals briefly with such attempts.



I



IIa, R = Ac
b, R = H

It was planned to degrade the ketol I to the corresponding allopregnane derivative II where troublesome interference with phosphorus tribromide was not anticipated, and to effect the removal of the 12-hydroxyl function at this stage with the formation of allopregnan-3β-ol-11,20-dione, a substance which has already been converted to cortisone.[7,8] In contrast to the unsatisfactory side chain degradation of 22a-5α-spirostan-3β-ol-12-one (hecogenin),[9] the corresponding reaction with the 11-isomer[10] proceeded rather readily and it is interesting to observe that a similar degradation of the 3,12-diol-11-one (I) recorded in the present paper gave equally satisfactory results. The intermediate $\Delta^{20(22)}$-5α-furostene-3β,12β,26-triol-11-one has already been described earlier,[6] but for optimum yields of Δ^{16}-allopregnene-3β,12β-diol-11-one diacetate it was neither necessary nor desirable to isolate this intermediate furostene derivative. The Δ^{16}-20-ketone diacetate was hydrogenated readily to the corresponding saturated analog (IIa) but all attempts to saponify this diacetate completely to the diol IIb—a necessary operation before selective acylation at C-3 and subsequent displacement at C-12 with phosphorus tribromide can be carried out—resulted in very poor yields. This disappointing result may well be due to two factors, the ready isomerization of the ketol system under basic conditions to a mixture of isomeric ketols[5] and possibly also partial isomerization at C-17 to the α-epimer, which may be favored by the 12β-hydroxy substituent. At the present time, therefore, the only successful conversion of hecogenin to cortisone still remains the one through 22a-5α-spirostan-3β-ol-11,12-dione.[2]

(1) Paper XXIV, M. Velasco, J. Rivera, G. Rosenkranz, F. Sondheimer and C. Djerassi, *J. Org. Chem.*, **17**, December (1952).

(2) Part 3, C. Djerassi, H. J. Ringold and G. Rosenkranz, THIS JOURNAL, **73**, 5513 (1951).

(3) Our sapogenin nomenclature (G. Rosenkranz and C. Djerassi, *Nature*, **166**, 104 (1950)) has been changed slightly in accordance with the recommendations of the Ciba Conference on Steroid Nomenclature (*cf. Chemistry and Industry*, June 23, 1951, SN1).

(4) Department of Chemistry, Wayne University, Detroit 1, Michigan.

(5) E. Borgstrom and T. F. Gallagher, *J. Biol. Chem.*, **177**, 951 (1949).

(6) C. Djerassi, H. Martinez and G. Rosenkranz, *J. Org. Chem.*, **16**, 303 (1951).

(7) J. M. Chemerda, E. M. Chamberlin, E. H. Wilson and M. Tishler, THIS JOURNAL, **73**, 4053 (1951).

(8) G. Rosenkranz, J. Pataki and C. Djerassi, *ibid.*, **73**, 4055 (1951); **74**, 5615 (1952).

(9) R. B. Wagner, J. A. Moore and R. F. Forker, *ibid.*, **72**, 1856 (1950).

(10) C. Djerassi, E. Batres, J. Romo and G. Rosenkranz, *ibid.*, **74**, 3634 (1952).