1959; Gual, Morato, Hayano, Gut & Dorfman, 1962) probably include a contribution due to the formation of oestrogen as an artifact from the 1β -hydroxy derivative, since base partitions were used.

Whether a similar situation exists with studies *in vivo* is not known. Although *in vitro* the 19-nor-steroids are relatively poor precursors of oestrogens, *in vivo* they, and their 17α -substituted derivatives, appear to be converted into oestrogens to an extent similar to or greater than testosterone (17β -hydroxyandrost-4-en-3-one) (Breuer, 1964; Dimick, Heron, Baulieu & Jayle, 1961; Engel, Alexander & Wheeler, 1958). Since the isolation and estimation procedures used in such studies involve acid hydrolysis and partition between aqueous base and organic solvent, the presence of 1β -hydroxylated derivatives would artificially inflate the value for oestrogens. For example, such a derivative could be the precursor of the oestrogenic artifact of 17α -ethynyl- 17 -hydroxyoestr-4-en-3-one that Brown & Blair (1960) suggest may be produced during the analysis of urine.

Owing to the increasing use of 19-nor-steroids as progestational agents, there is an active interest in their metabolism. The present communication provides evidence for the possibility of producing 1β -hydroxy metabolites that, in investigations of oestrogen biosynthesis, make contributions of artifacts unless adequate precautions are taken.

Stereochemical studies and the cofactor requirements for aromatization of C_{18} and C_{19} steroids suggest the possibility of 1β -hydroxylated androgens' being intermediates in aromatization (Morato *et al.* 1962; Townsley *et al.* 1966). However, neither

the isolated radioactive 1β -hydroxy-19-norandrostenedione nor the authentic non-radioactive compound was converted into oestrogens by the placental system under conditions where appreciable amounts of oestrogens were formed from 19-norandrostenedione.

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The Biotransformation of Gramine into Tryptophan in Barley Shoots

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The alkaloid gramine (VI) is formed in germinating barley from tryptophan (Bowden & Marion, 1951a,b). Wenkert (1962) suggested that a key intermediate in the above biotransformation is a protonated 3-methyleneindolenine species (IV) (Scheme 1), which could arise by a reverse Michael degradation of the Schiff base (III) formed by the

interaction of tryptophan (I) and pyridoxal phosphate (II). Attack of the intermediate (IV) by ammonia, followed by methylation, could lead to gramine (VI). Wenkert's (1962) hypothesis has been supported by experiments *in vivo* (O'Donovan & Leete, 1963; Gower & Leete, 1963) and by amine fragmentation *in vitro* (Whitlock & Digenis, 1964).

Scheme 1.

When gramine is heated or treated with base it loses its amino function, yielding products that are believed to arise via the indolenine (IV) (Snyder & Eliel, 1948; Leete & Marion, 1953). Since gramine (normally absent from the roots) disappears from barley shoots after the fiftieth day after germination (Tyler, 1958) it appeared to us that this alkaloid could possibly revert back to the indolenine (IV) which could then be attacked by a glycine moiety (possibly in its azomethine form, V), to give tryptophan. The present communication shows that when 60- and 120-day-old barley were provided with labelled gramine, the alkaloid was incorporated into the tryptophan fraction of the plant. No other amino acid from the barley shoots was found to be radioactive.

Methods. A native strain of Lebanese barley (designated as Baladi 25, Telamara) attributed to *Hordeum distichon* L. was grown in a botanical garden exposed to normal atmospheric conditions. The shoots of this strain 17 days after germination have been analysed by the method of Gower & Leete (1963) and found to contain 0.37% (w/w) of

gramine (on dried-herb basis) by Digenis, Abou-Chaar & Helou (1966).

Gramine, labelled in the carbon attached to the ring, was synthesized from indole, dimethylamine and [^{14}C]paraformaldehyde (The Radiochemical Centre, Amersham, Bucks.) by using the procedure of Kühn & Stein (1937). The radioactive gramine was sublimed twice and crystallized once from *n*-hexane and was shown to be pure by mixed melting point with authentic gramine, ultraviolet spectroscopy and thin-layer chromatography. Only one radioactive spot was detected by radioautography of the chromatograms in two solvents.

All radioactivity measurements were performed in a liquid-scintillation counter with a background count of 12 counts/min. and an efficiency of 70%. All experiments were run in duplicates.

Results. Barley shoots, cut very close to the grain, were analysed for their gramine content (Gower & Leete, 1963) every fourth day. At the fiftieth day only traces of gramine could be detected (thin-layer chromatography). When a 60-day-old sample of the shoots was defatted and analysed (Wightman,

Chisholm & Neish, 1961) it was found to contain 0.28% (w/w) of tryptophan (on the basis of the defatted weight).

In a representative experiment 3mg. of labelled gramine (501 590 counts/min./mg.) dissolved in 3ml. of 5M-N-acetic acid was supplied to 12 60-day-old shoots by using the wick-feeding technique of Leete (1965). These were harvested 7 days later and dried at 50° and 24 hr. The method described below is mainly that of Wightman *et al.* (1961).

The defatted plant material (3.77 g.) was subjected to alkaline hydrolysis (20%, w/v, NaOH) at 110° in a sealed tube for 24 hr. The digested material was filtered and the filtrate after adjustment to pH 6.0 was concentrated to 20ml. *in vacuo*. The concentrate, containing the amino acids, was adsorbed on a 1.5 cm. x 40 cm. column of Amberlite IR-120 (H⁺ form) ion-exchange resin. The column was washed with 300ml. of distilled water, and the amino acids were eluted with 250ml. of 1.5N-NH₃ and subsequently evaporated to dryness under reduced pressure. The residue containing the total plant amino acids was dissolved in 10.0ml. of 0.5N-acetic acid and a sample was counted. The total sample was found to contain 12 800 counts/min. To a 2ml. sample from the amino acid mixture containing 2mg. of tryptophan and 2560 counts/min., 100mg. of carrier tryptophan was added and the whole mixture was evaporated to dryness. The tryptophan was recrystallized three times from dilute ethanol, once from acetic acid and twice from methanol to constant specific activity of 21.7, 23, 22, 25, 28 and 26 counts/min./mg. (calculated 25 counts/min./mg.). Another sample (30 µl.) from the amino acid mixture was subjected to two-dimensional paper chromatography with carrier tryptophan (30 µl. of 0.1% solution). The tryptophan spot was located with an ultraviolet lamp, cut out and counted. It was found to contain 40 counts/min. If all the radioactivity of the amino acid mixture were in tryptophan then the expected count would be 38.4 counts/min. The rest of the spots (including that from the origin) from the chromatogram were eluted, counted and found to contain no radioactivity. Serine, of especial interest, was also unlabelled. Very little radioactive gramine was recovered (2300 counts/min.) from the beakers and threads that constituted the wicks by which the plants absorbed the labelled alkaloid.

Practically no labelled gramine could be recovered (114 counts/min.) from a batch of 12 60-day-old shoots similarly provided with gramine and harvested at the same time.

Discussion. From the above results it was calculated that 0.84% of the gramine was converted into tryptophan in the 60-day-old barley. When the experiment was repeated with 120-day-old barley it was found that 0.5% of gramine was incorporated into tryptophan.

The fact that no serine was found labelled suggests that gramine was not degraded in the plant to dimethylamine, formaldehyde and indole. Thus it does not appear that the route by which the alkaloid incorporated tryptophan is the same as the well-known biogenetic pathway to this amino acid from indole and serine. A possible route is the reverse of that shown in Scheme 1. Efforts are being made in our Laboratory to establish the intermediacy of the indolenine (IV), since this species is believed to be involved in hydrogen transfer reactions catalysed by yeast alcohol dehydrogenase (Schellenberg, 1965).

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